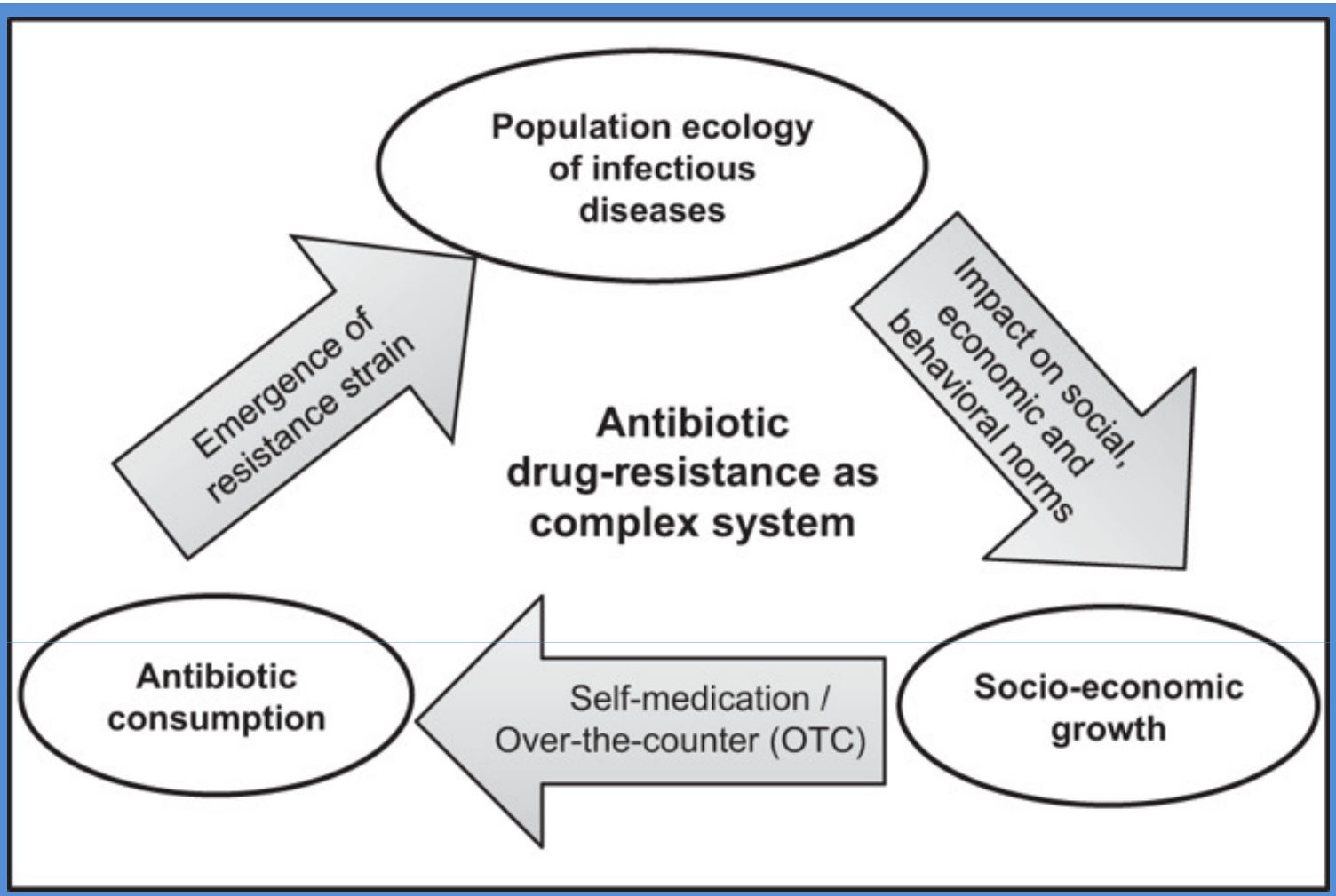
Romeo T. Cristina®

Antimicrobial resistance and prudent use of antimicrobials in veterinary medicine

C.4-5. / S.II.



- **1. Economical and social impact of resistance**

- **The widespread use** of antimicrobial substances in both human and veterinary medicine has increased

→ *acceleration in the occurrence and spread of AMR*

- According to literature, **the amount of veterinary antibiotic consumed worldwide by livestock is almost double** that of human use.

- For example, in USA

for obtaining **1 kilogram of meat and eggs**

300 mg of the antibiotic

- In addition to the **therapeutic or preventive use** of antimicrobial substances, in many countries they are used as **growth promoters**.
- In the late 1940s - farmers administered antibiotics to livestock

→ **resistant bacterial strains**

- **The European Union banned the use of antibiotics as a growth promoter in 2006**

- In general, there are **convictions that without antibiotics the livestock industry will collapse**
- Some authors and countries support and demonstrate the opposite, for example:
- **In Denmark**, the largest European country exporting pork in the world, exporting about 90% of what they produce, there has been an increase after banning the use of antibiotics for raising pigs

Comment | Published: 27 June 2012

Sustainable farming

Get pigs off antibiotics

Frank Aarestrup

Nature **486**, 465–466(2012) | Cite this article

1308 Accesses | **74** Citations | **114** Altmetric | Metrics

Frank Aarestrup explains how he helped Denmark to cut the use of antibiotics in its livestock by 60%, and calls on the rest of the world to follow suit.

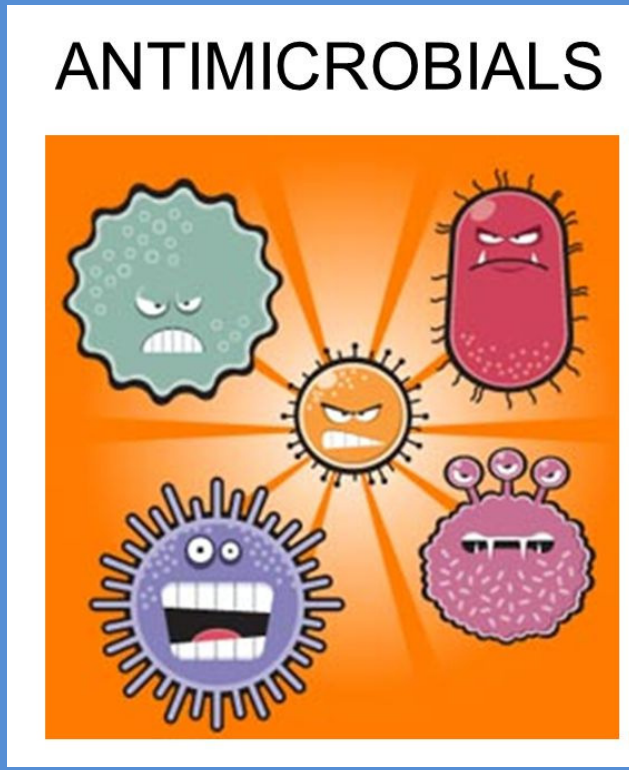
- **In 2008,** following a European Council debate on antimicrobial resistance, **EU Member States decided:**

strengthening surveillance systems

improving the quality of data on antimicrobial consumption and resistance, both in the human and veterinary sectors.

- The European Commission has asked the European Medicines Agency (EMA) to take the lead in collecting data on the use of antimicrobial agents for veterinary use in the Member States. EMA has requested to consult:
- **the European Center for Disease Prevention and Control (ECDC),**
- **the European Food Safety Authority (EFSA)**
- **the EU Reference Laboratory for Antimicrobial Resistance (EURL-AMR)**

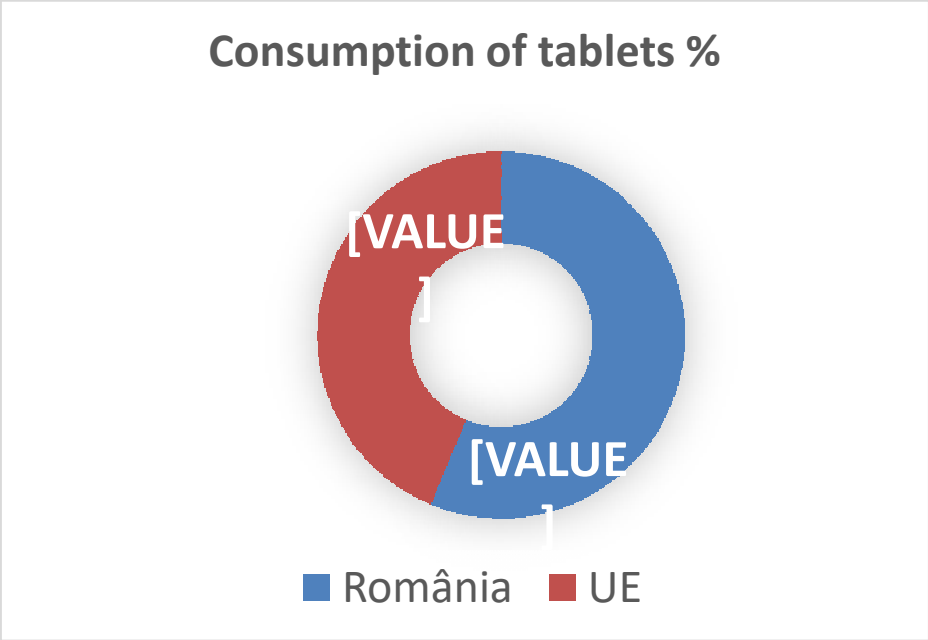
Consumption…

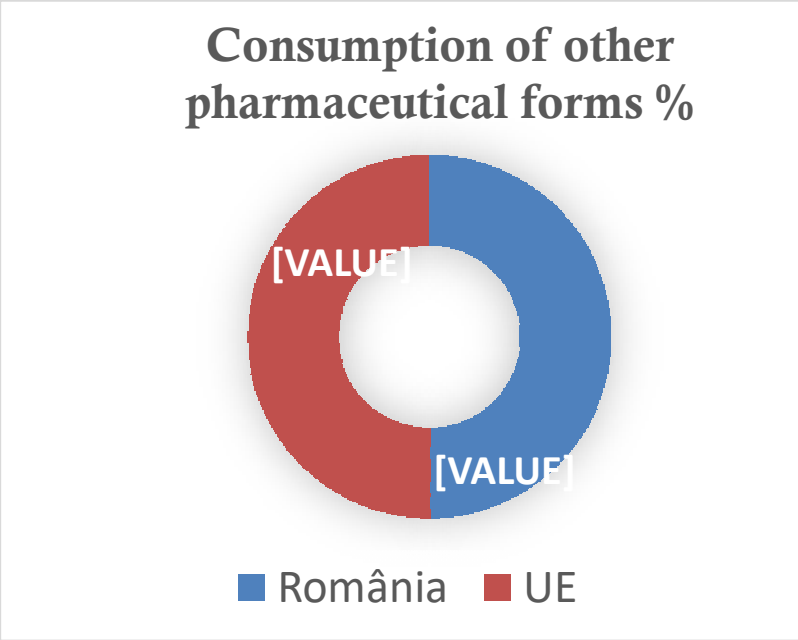


According to EMA-*Trends from 2010 to 2015*
Seventh ESVAC report ***in Romania***

- The consumption of antimicrobials for veterinary use was 259.6 tonnes of the active substance, of which 257.2 tonnes of active substance were medicinal forms administered mainly to animals of income (99.1% of the total), and only 2.4 tonnes of the active substance in the form of tablets administered mainly to pets

	Tablets		Other pharmaceutical forms		
Country	Tonne	Percentage of total consumption	Tonne	Percentage of total consumption	Total tone
Romania	2.4	0,9%	257.2	99,1%	259.6
Average EU / 30 countries	2.08	0.7%	276.63	99,3%	278.71





According to EMA-Trends from 2010 to 2015 Seventh ESVAC report in the 30 participating countries

- The consumption of veterinary antimicrobial preparations, expressed in mg per unit of population correction (PCU), ranged from 2.9 mg / PCU to 434.2 mg / PCU.
- Also the use of antimicrobial classes varied substantially depending on the country

	Consumption (tonnes) for livestock	PCU (1.000 tonne)	mg/PCU
Romania	**257.2**	**2.559**	**100.5**
EU	**276.63**	**312.9**	**100.6**

Source:
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/580710/1101060-v1-Understanding_the_PCU_-_gov_uk_guidance.pdf

What is PCU = Population Correction Unit?

Slaughter cows	425 kg
Slaughter heifers	200 kg
Slaughter bullocks and bulls	425 kg
Slaughter calves & young cattle	140 kg
Imported/exported cattle for slaughter	425 kg
Imported/exported for fattening	140 kg
Livestock dairy cows	425 kg
Slaughter pigs	65 kg
Imported/exported pigs for slaughter	65 kg
Imported/exported pigs for fattening	25 kg
Livestock sows	240 kg
Slaughter broilers	1 kg
Slaughter turkeys	6.5 kg
Imported/exported poultry for slaughter	1 kg
Slaughter sheep & goats	20 kg
Imported/exported sheep & goats for slaughter	20 kg
Livestock sheep	75 kg
Living horses	400 kg
Slaughtered fish based on liveweight	- - kg
Slaughter rabbits	1.4 kg

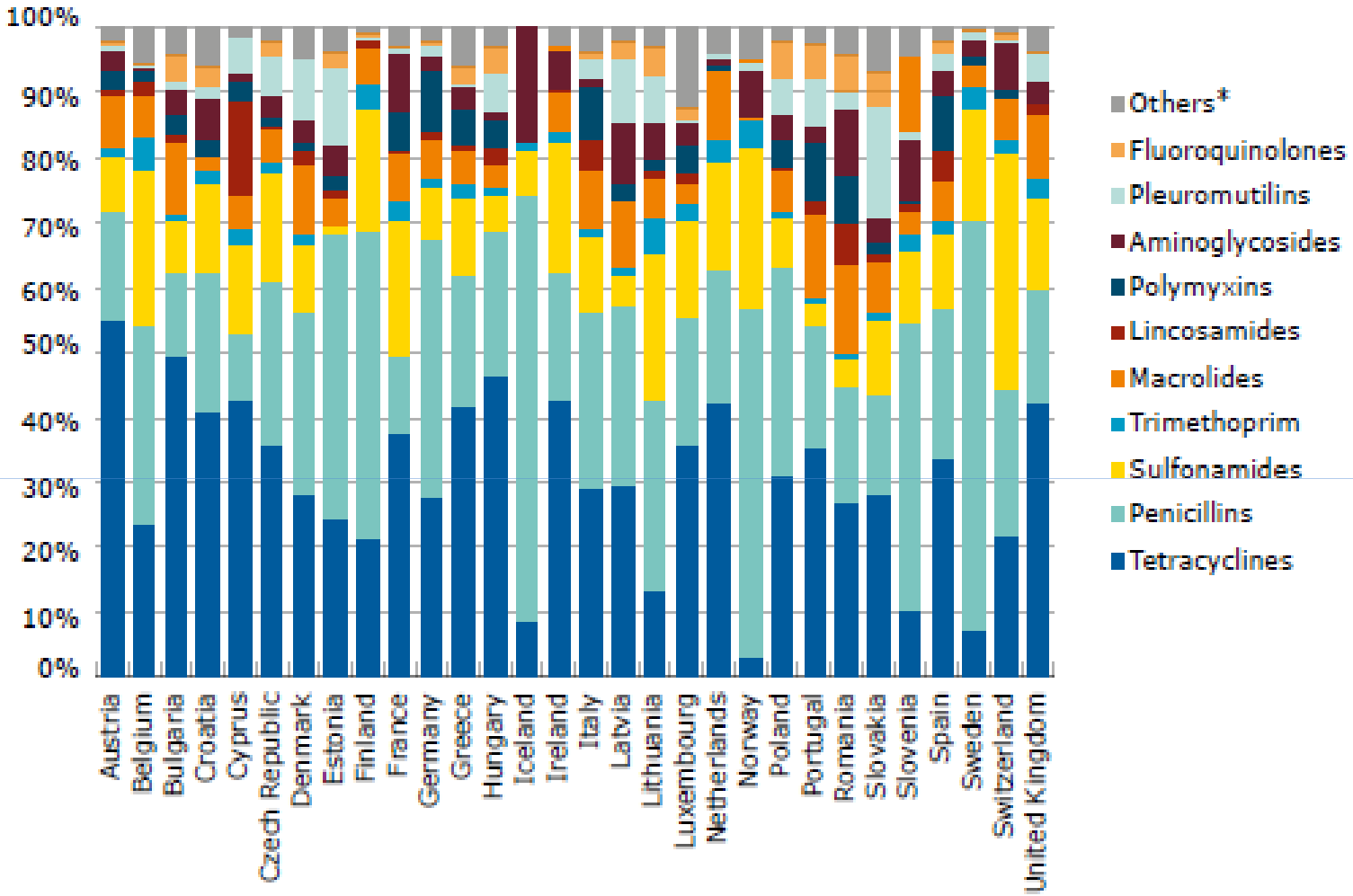
Understanding the Population Correction Unit used to calculate antibiotic use in food-producing animals.

According to the data, in Europe, during the short existence:

- a chicken is treated with antibiotics 2.3 times
- 5.3 times a pig!

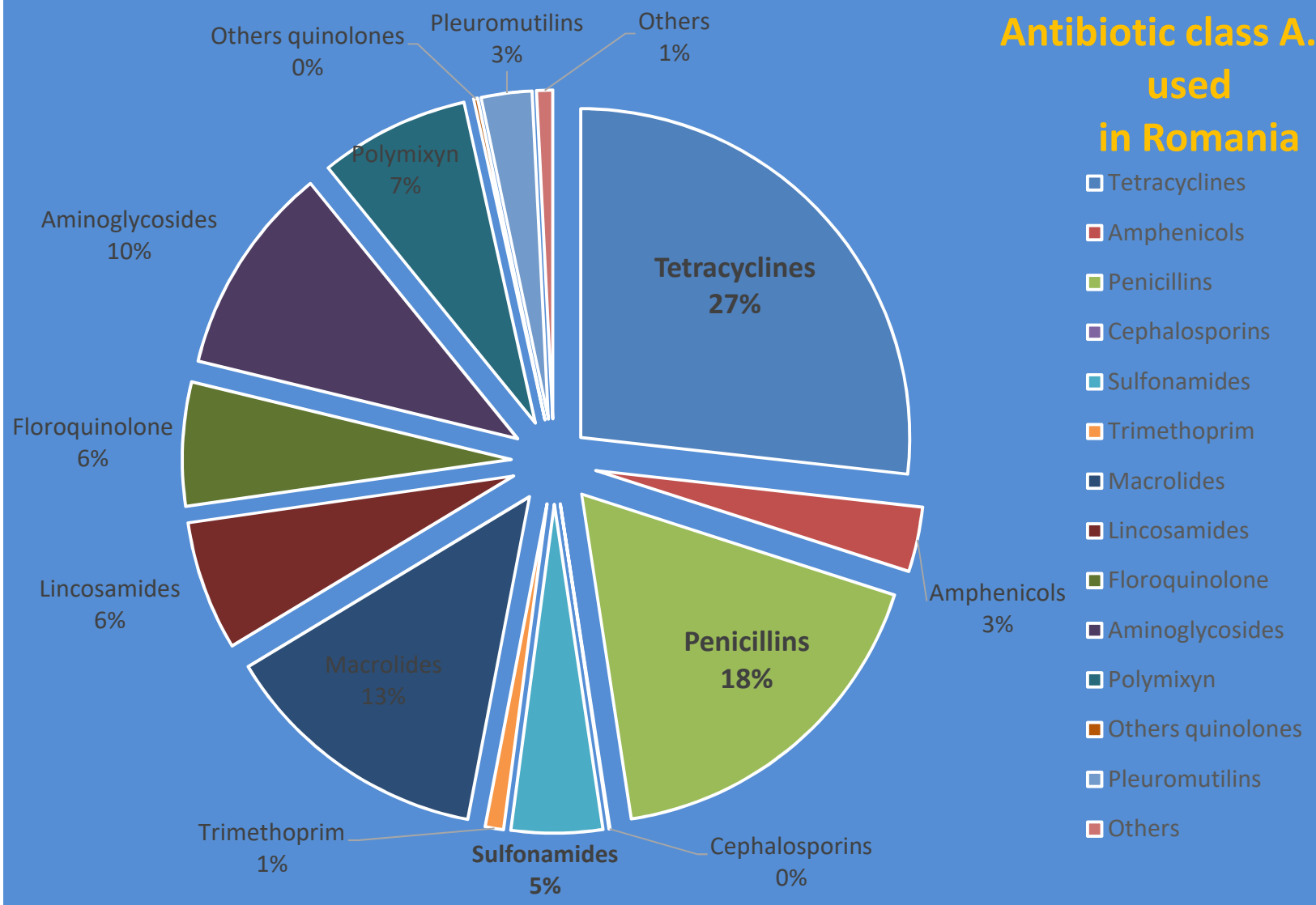
- In Romania, the main indicator for antimicrobial consumption, namely mg / PCU, which indicates the amount of active substance consumed equivalent by the population correction unit, had a value of 100.5 mg / PCU, being almost identical to the average (100.6 mg (PCU) from the EU.
- The highest values for this indicator were recorded in Cyprus and Spain with values of 434.2, respectively 402.0 mg / PCU

Proportion of the total sales of the different veterinary antimicrobial classes, in mg/PCU, in the 30 European countries, for 2015



Source:
https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-resistance-europe-2015.pdf

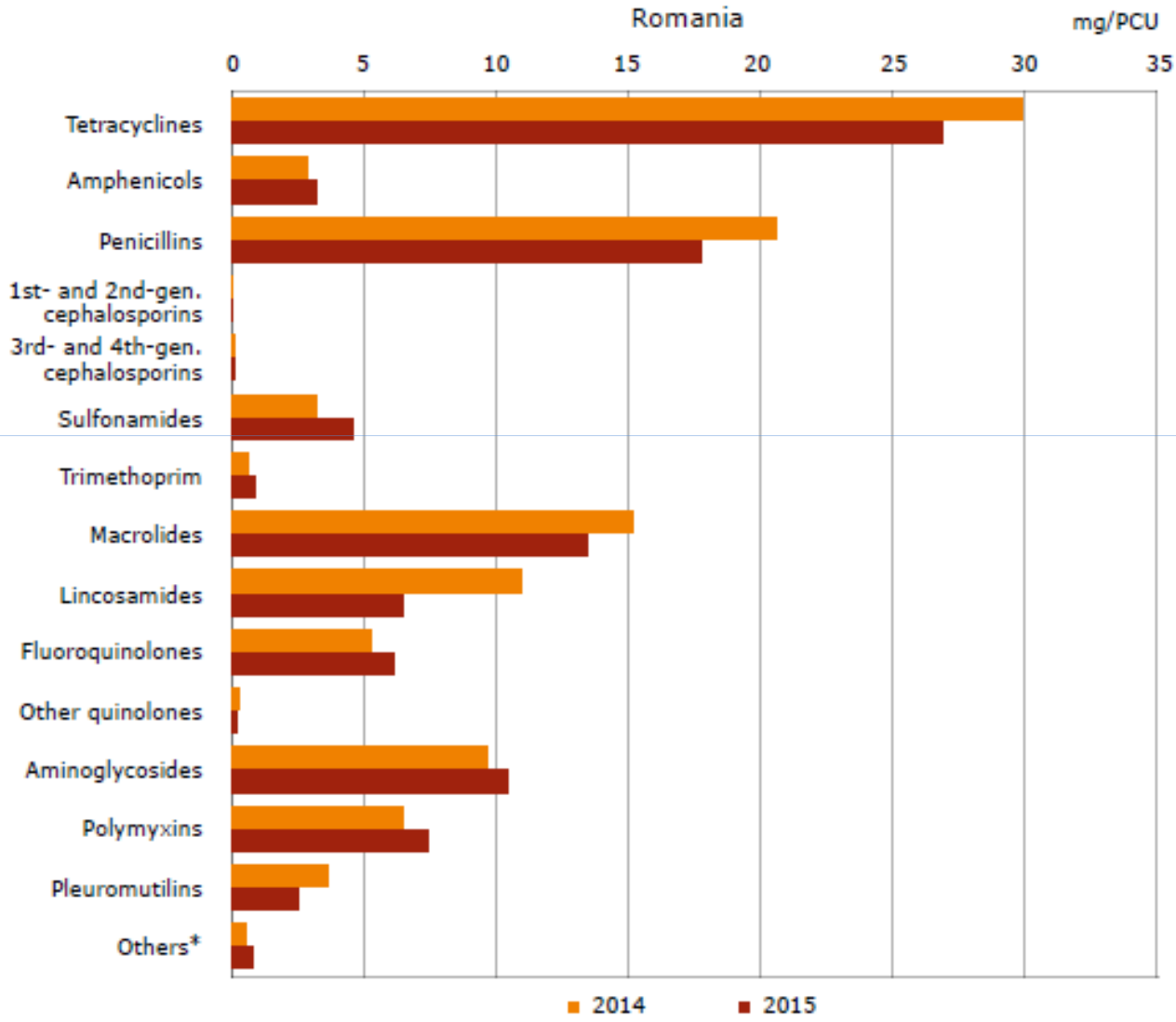
In Romania the consumption of tetracyclines represented the highest value 27%, and together with penicillins and sulfonamides they represented 50% of the total amount of antimicrobials used (100.5 mg / UPC).



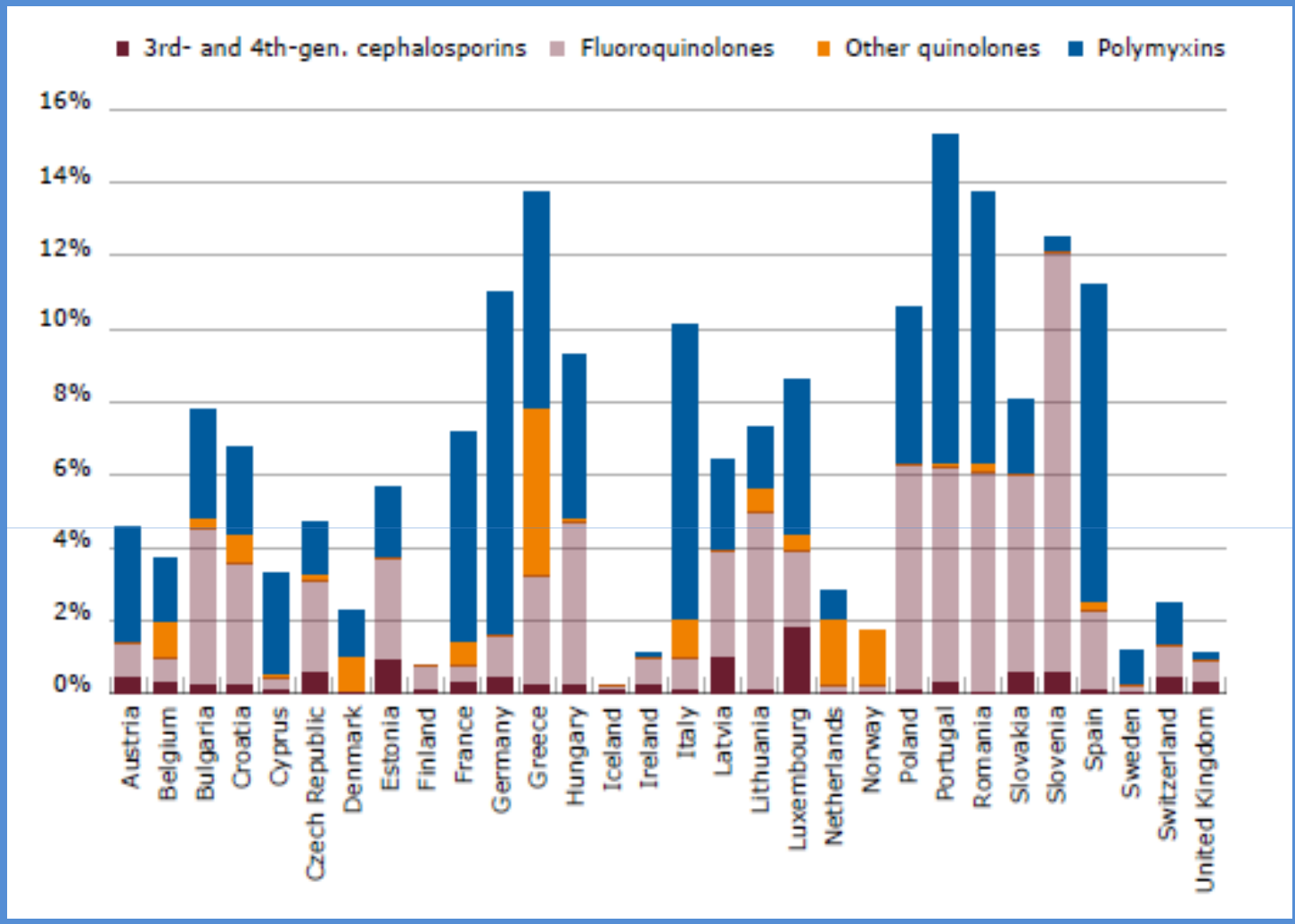
According to EMA-Trends from 2010 to 2015 Seventh ESVAC report

Romania

Figure 105. Sales (mg/PCU) by antimicrobial class in Romania, from 2014 to 2015



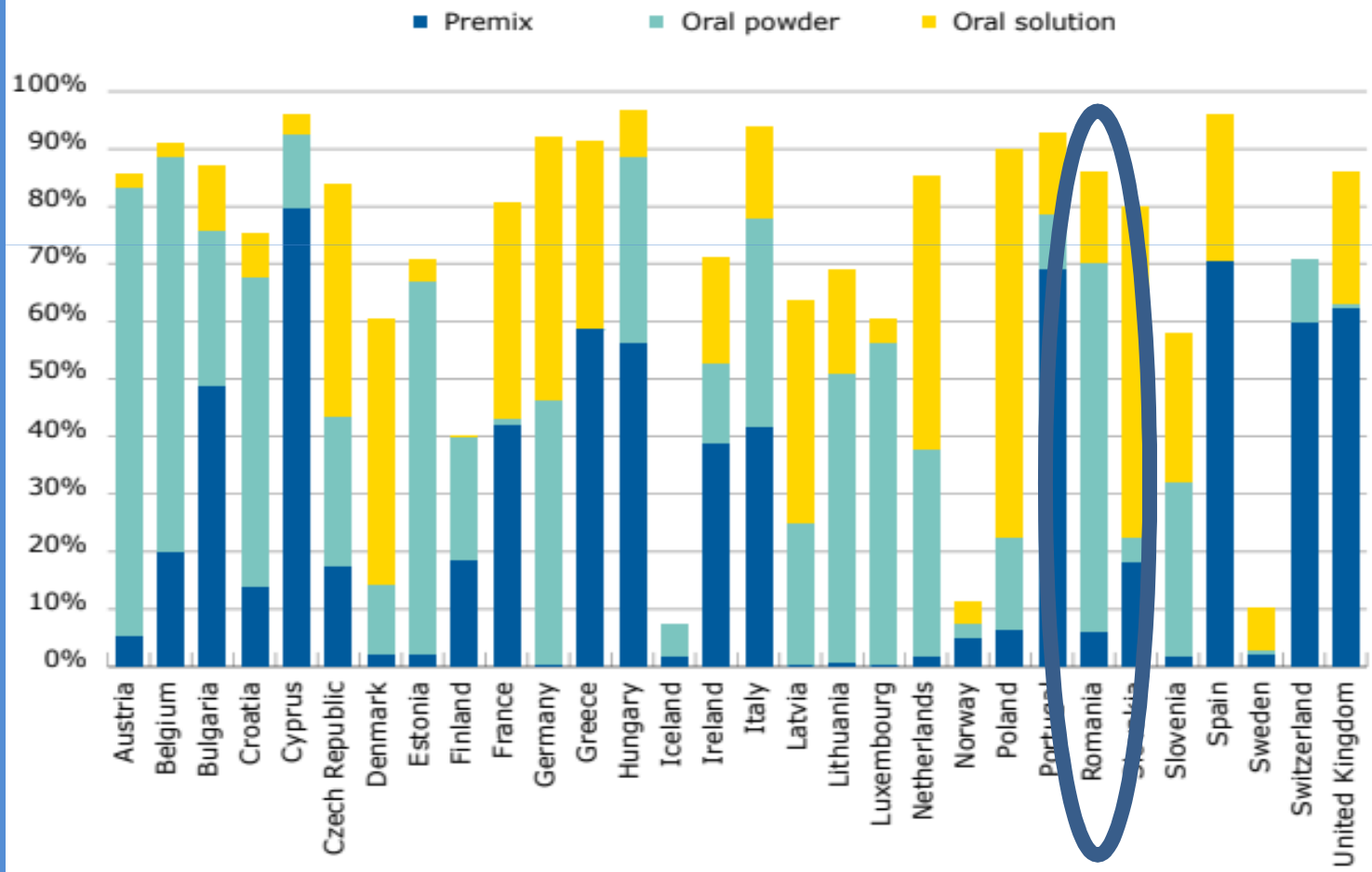
Source: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-resistance-europe-2015.pdf



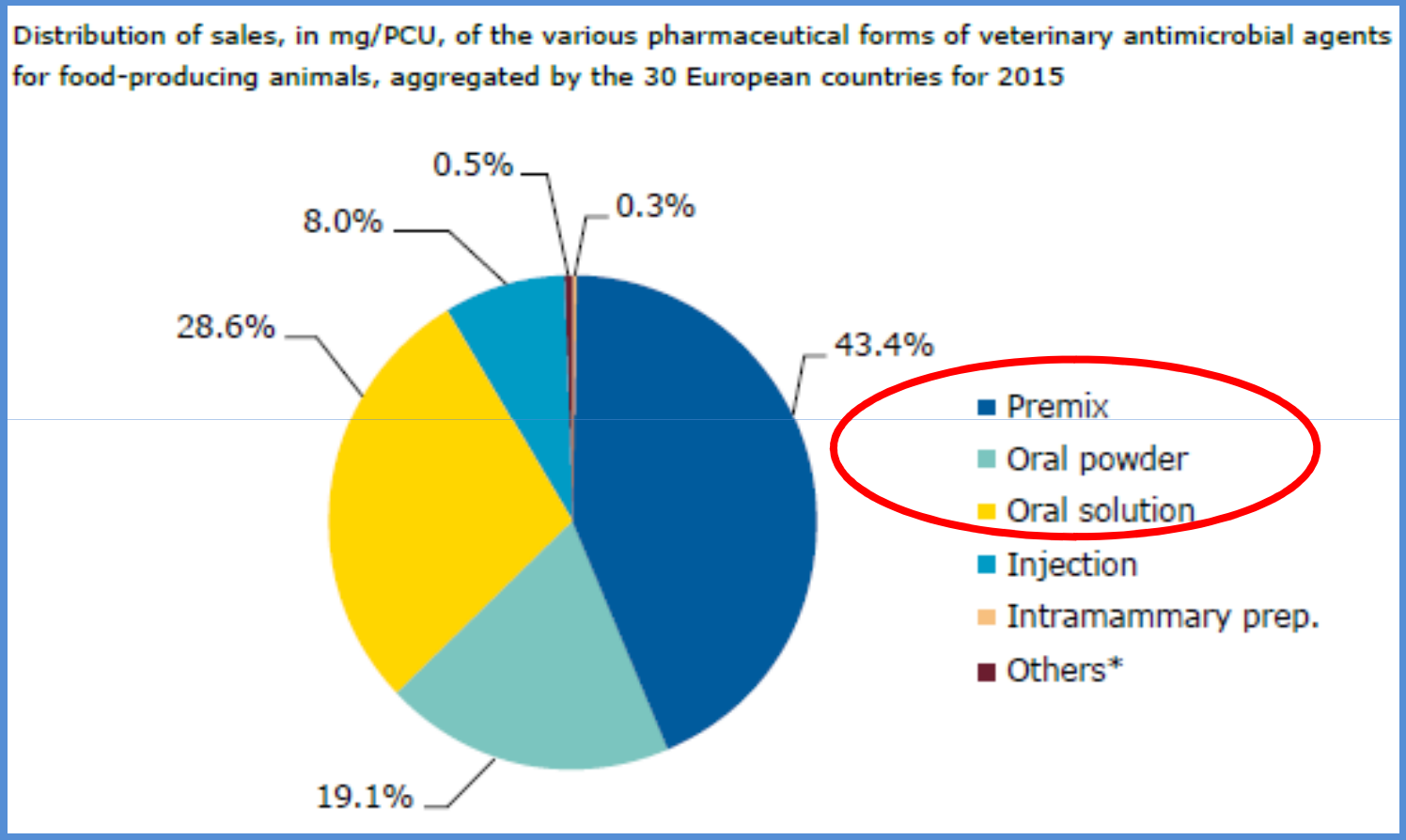
Source:
https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-resistance-europe-2015.pdf

In Romania, the most widely used forms of antibiotic administration in the livestock were: oral powders, oral solutions and premixes. These three categories represent about 87% of all forms

Oral solutions, oral powders and premixes as percentages of total sales, in mg per population correction unit (mg/PCU), of veterinary antimicrobial agents for food-producing animals, in 30 European countries for 2015



In EUROPE



Source:
https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-resistance-europe-2015.pdf

In: ***Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report.***

Published: EFSA Journal 2017;15(7):4872, 135 pp. doi:10.2903/j.efsa.2017.4872

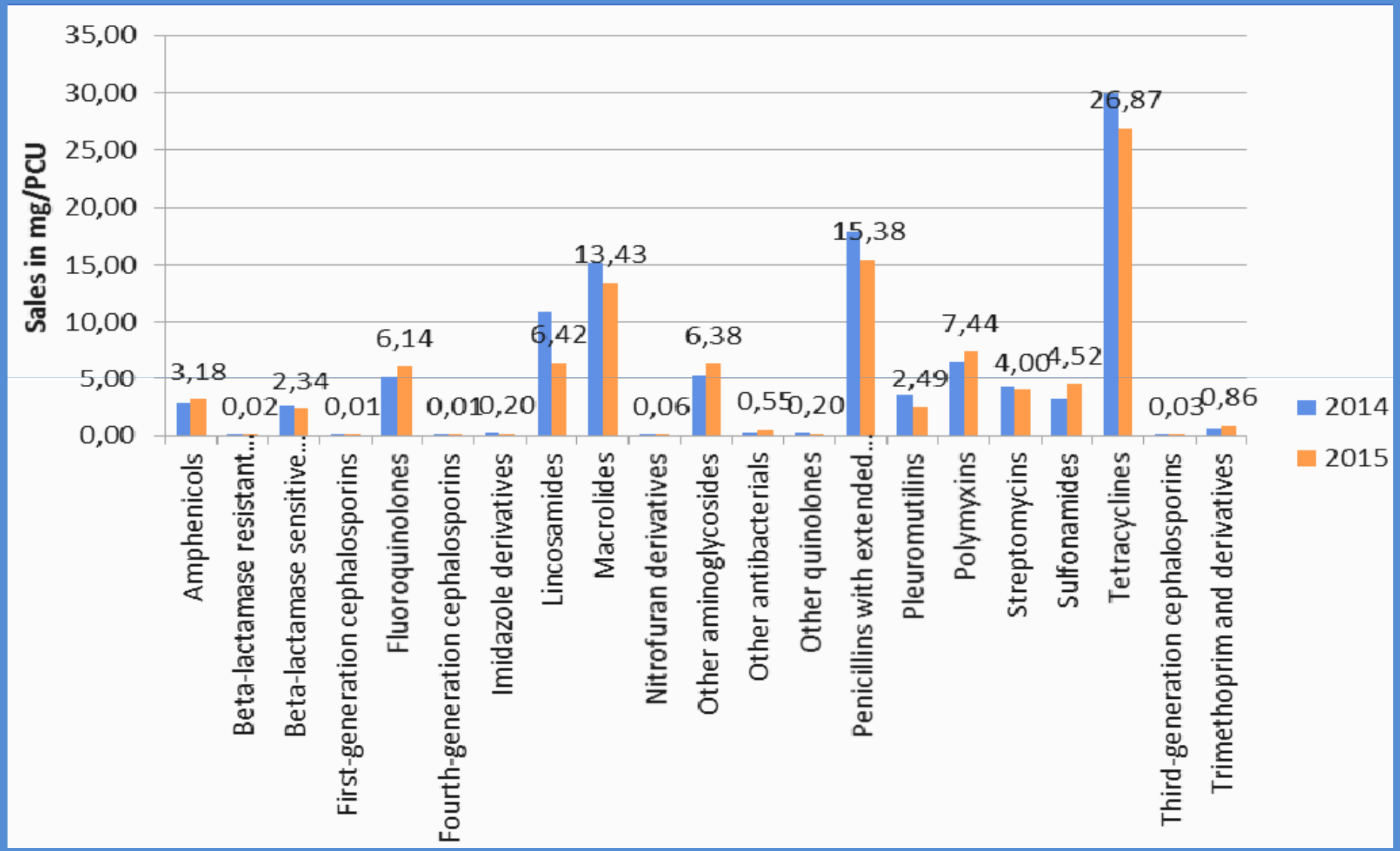
50 mg/kgc.

Over a three-year period 2013-2015, the estimated total average consumption of antimicrobials expressed in mg s.a / kgc live animal was:

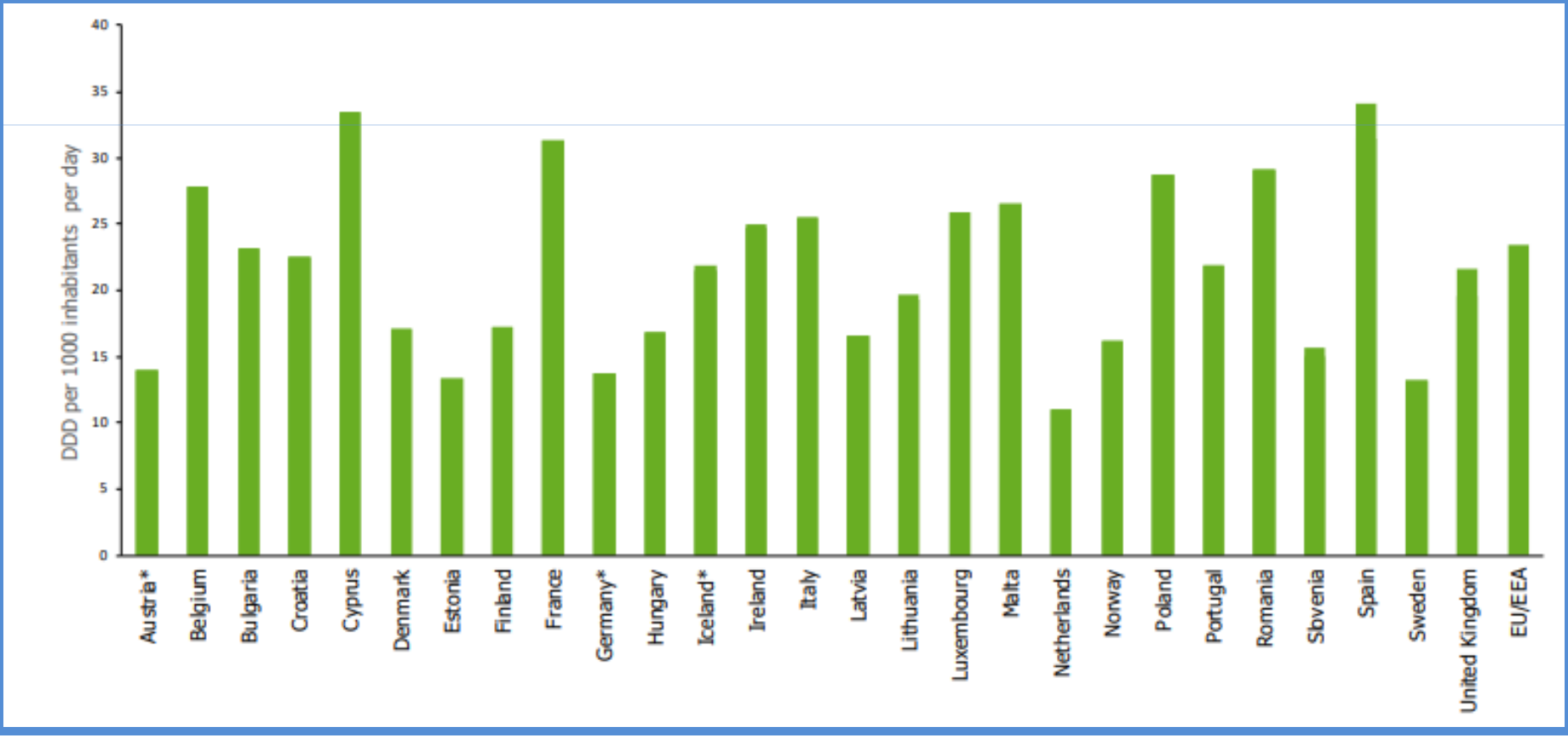
124 mg / kgc in humans = average 118 mg / kgc. (50-182 mg / kgc) and 152 mg / kgc in livestock = average 67 mg / kg; (3-419 mg / kgc).

Antibiotic consumption rates were lower or much lower in animals, in 18 of 28 countries, in 2 countries it was similar, and in 8 the consumption was much higher for animal vs human.

Romania (compare 2014 / 2015)



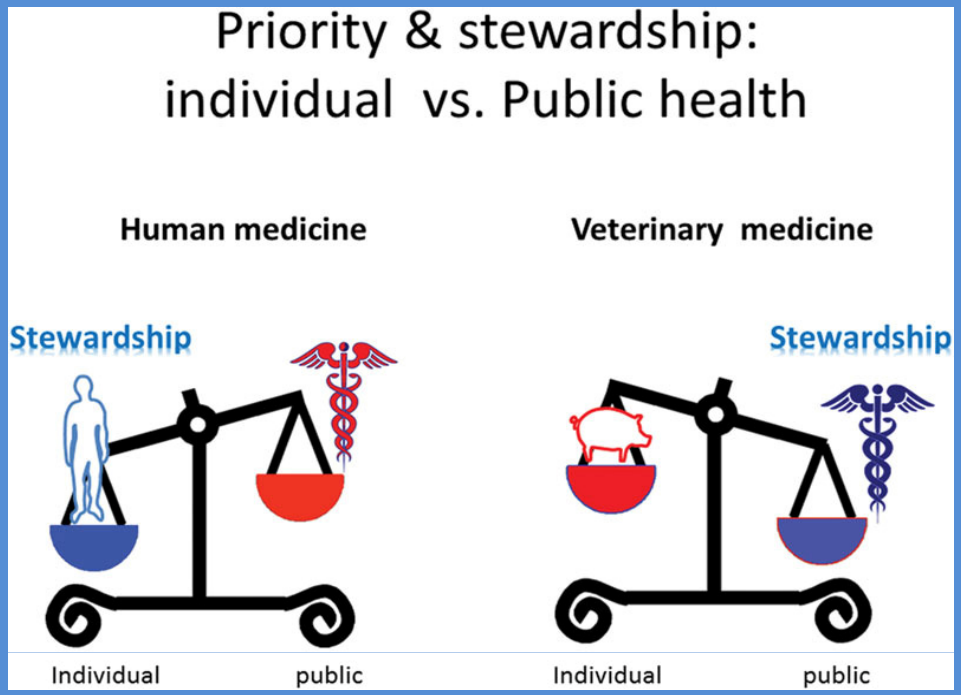
- **According to ECDC și EFSA**
- In Romania, the high consumption of antimicrobials in both **human medicine, which was 29.1 DDD / 1,000 inhabitants,** and **in veterinary medicine, 257.2 tonnes or 100.5 mg / PCU,** ranks us among the first places at European level.



Impact
of reckless use of anti-infectives

... considerable and difficult to calculate!

Fact



The consumption of drugs in veterinary medicine exceeds that of human medicine and it is recognized that veterinary medicine contributes majorly to the emergence and spread of resistance in humans ... (WHO, 2012).

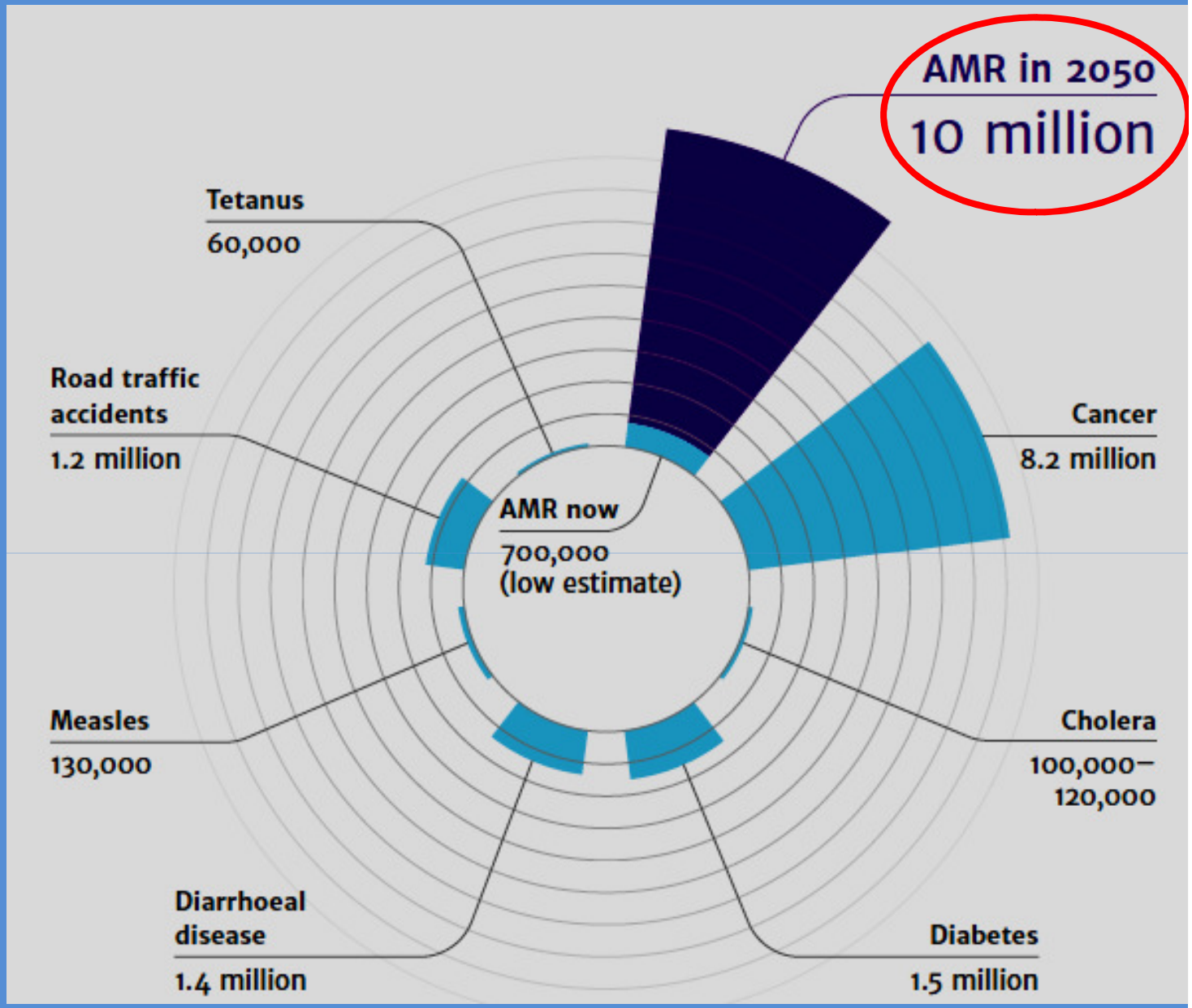
- **By: Jim O'Neill în:** *The review on antimicrobial resistance 2016 / Tackling Drug-resistant Infections Globally: Final Report and Recommendations*

https://amrreview.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

- More than 70% of the bacteria that cause intra-hospital infections are resistant to at least one antibiotic!
- In the world it is estimated that every year, drug-resistant infections result in the death of at least 700,000 patients and generate costs of 1.5 billion euros/year in the EU alone, hence the phenomenon of resistance (especially to antimicrobials)

became one of the priorities of C.E.

- About 200,000 people / year die from multi-resistant antibiotic strains!
- In India, antibiotic-resistant neonatal infections are the cause of death of approx. 60,000 newborns / year!
- Of the 40,000,000 people / year who are treated with antibiotics for respiratory diseases, only 13,000,000 have confirmed the need through laboratory examinations!
- The economic impact is considerable and difficult to calculate.
- In the US alone, more than 2 million infections / year are the result of bacterial resistance to first-line antibiotics, treatments, costing the US $ 20 billion extra health system every year!

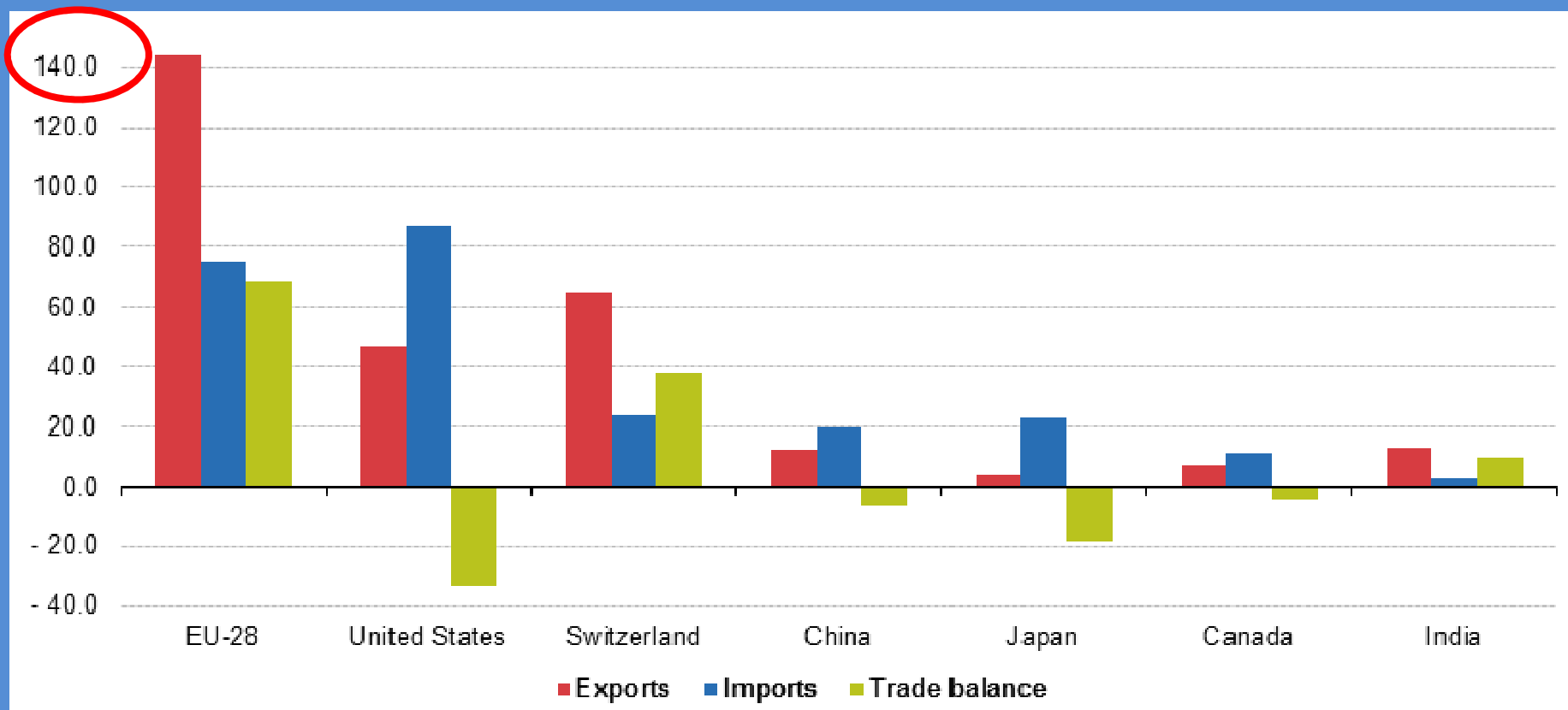


Source: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Citing ECDC and EMA sources in the EU:

The consequences of the resistance phenomenon are dramatic:

- multi-resistant strains: approx. 400,000 strains / year;
- additional days of hospitalization: 2,500,000 days of hospitalization / year;
- out-of-hospitalization costs: 900 million euros / year;
- productivity losses: 600 million euros/year.
- annually, about 4 million patients acquire an infection associated with healthcare.

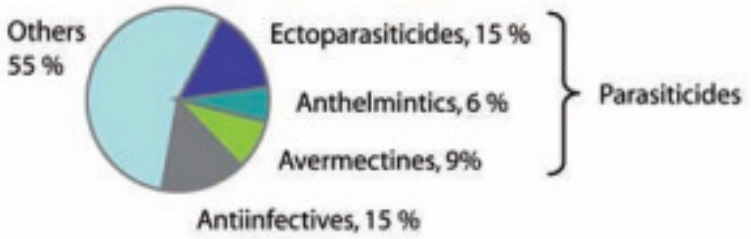


2016

Source:
EUROSTAT

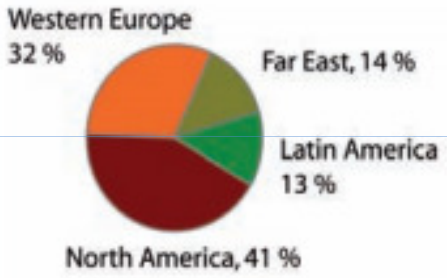
http://file.zums.ac.ir/ebook/072-Antiparasitic%20and%20Antibacterial%20Drug%20Discovery%20-%20From%20Molecular%20Targets%20to%20Drug%20Candidates%20(D.pdf

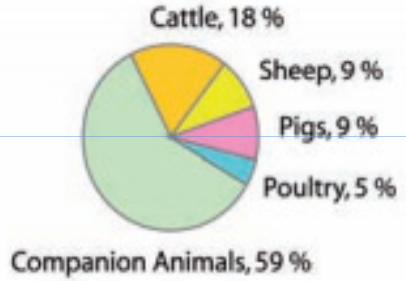
Animal Health Market: 16 Billion USD

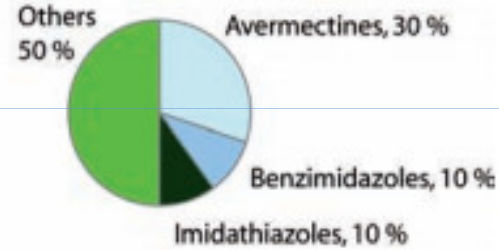


2006

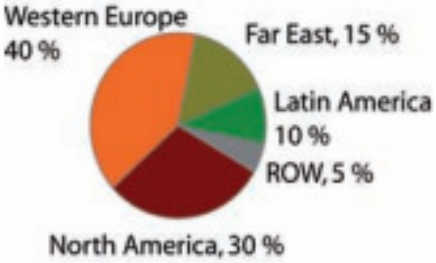
Combined Parasiticides Market: 4.8 Billion USD

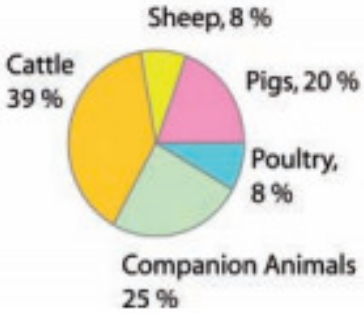


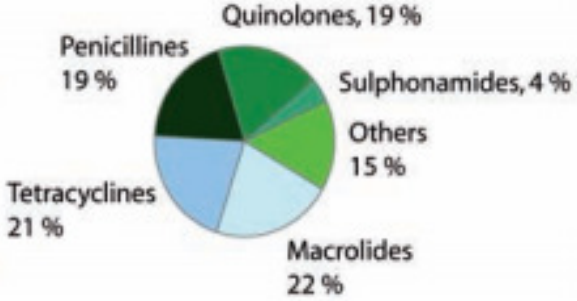




Antiinfectives Market: 2.4 Billion USD







*AMR is **not** a problem that can be solved **by any one country**, or even any one region... Our broad estimate for the cost of taking global action on AMR is up to **40 billion USD** over a **10-year period** (Jim O'Neil, 2016)*

!

2014 **800** *(oncogene)*

50 *(antibiotics)*

prevention

4.5 / an

60

U S N I H

1.2% *antibio*

18,6% *onco*

UK: Swine flu = $ 1.9 billion / year

US: Ebola = $ 5.4 billion / year

30.000-70.000 tonne

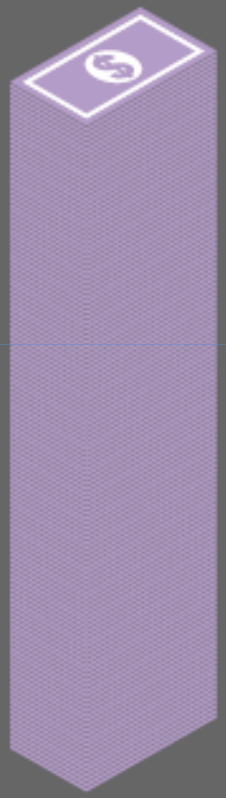
10-20%

!

200

Less than 5%

of venture capital investment in pharmaceutical R&D between 2003 and 2013 was for antimicrobial development.



Total venture capital investment

$38bn

Antimicrobial venture capital investment

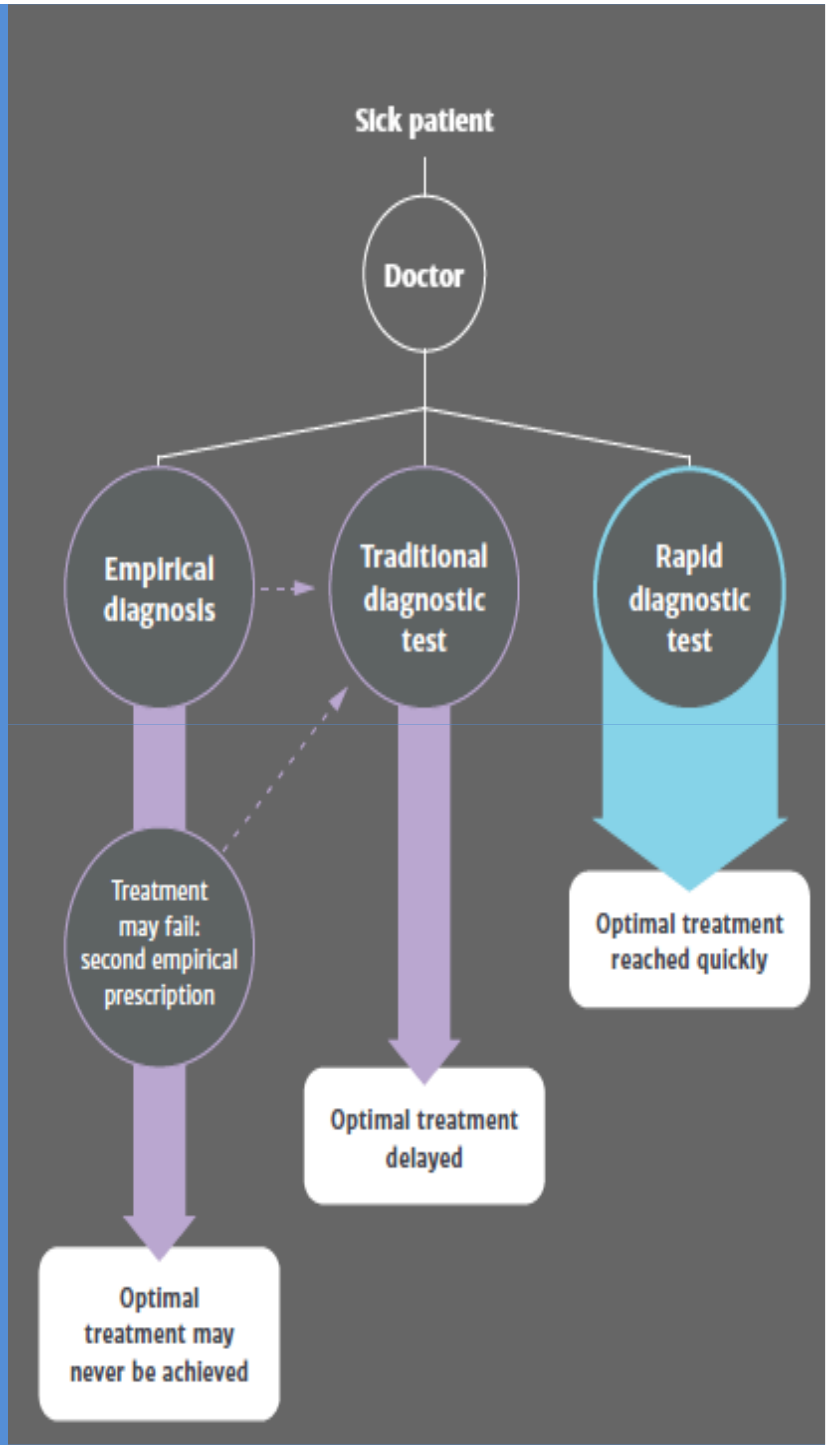
$1.8bn

Source: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Out of 40,000,000 Americans
taking antibiotics for
respiratory problems/year
27.000.000 take them for nothing!

*Today, **antibiotics are rarely prescribed based on a definitive diagnosis**. Diagnostic tests can show whether or not an antibiotic is actually needed, and which one.*

Dr Margaret Chan,
Director General of the WHO



Fact!

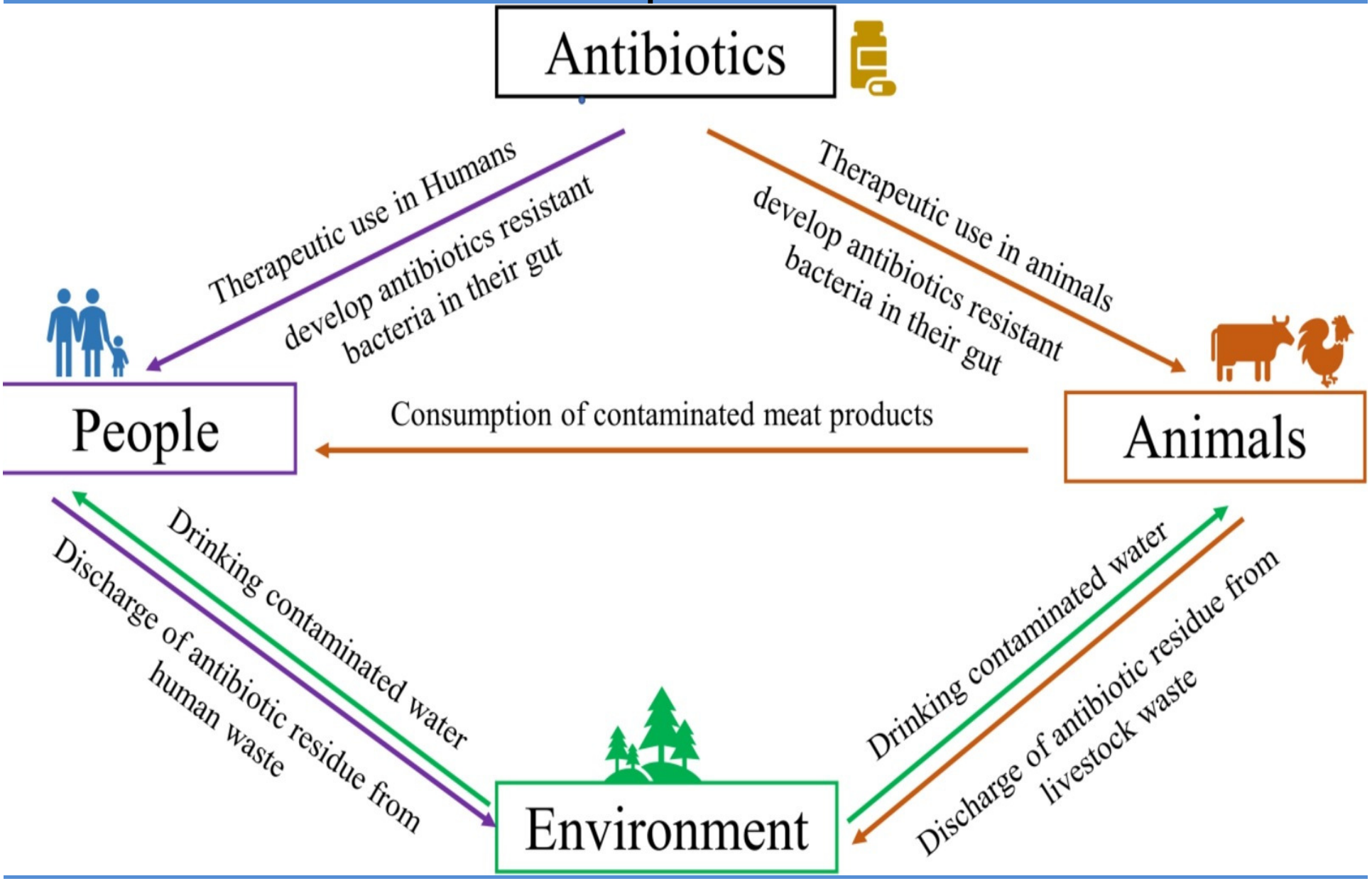
Organ / țesut	Cal	Câine	Capră	Vacă	Om
Sânge	8,60	-	4,70	7,80	-
Creier	0,21	0,51	0,29	0,06	2,00
Cord	0,66	0,82	0,48	0,37	0,47
Pulmon	0,89	0,89	0,88	0,71	1,40
Ficat	1,30	2,32	0,95	1,22	2,60
Splină	1,11	0,26	0,25	0,16	0,26
Rinichi	0,36	0,61	0,35	0,24	0,44
Intestine	5,80	3,90	6,40	3,80	1,70
Continut intestinal	12,30	0,72	13,90	18,40	1,40
Piele	7,45	9,30	9,20	8,30	3,70
Mușchi	40,10	54,50	45,50	38,50	40,00
Oase	14,60	8,70	6,30	12,70	14,00
Tendoane	1,71	-	-	-	2,00
Tesut adipos	5,10	-	-	18,09	18,10
Greutate medie	380,0	16,0	39,0	620,0	70,0

Proporția distribuției în diferite organe și tesuturi (Sinteză, Cristina, 2000)

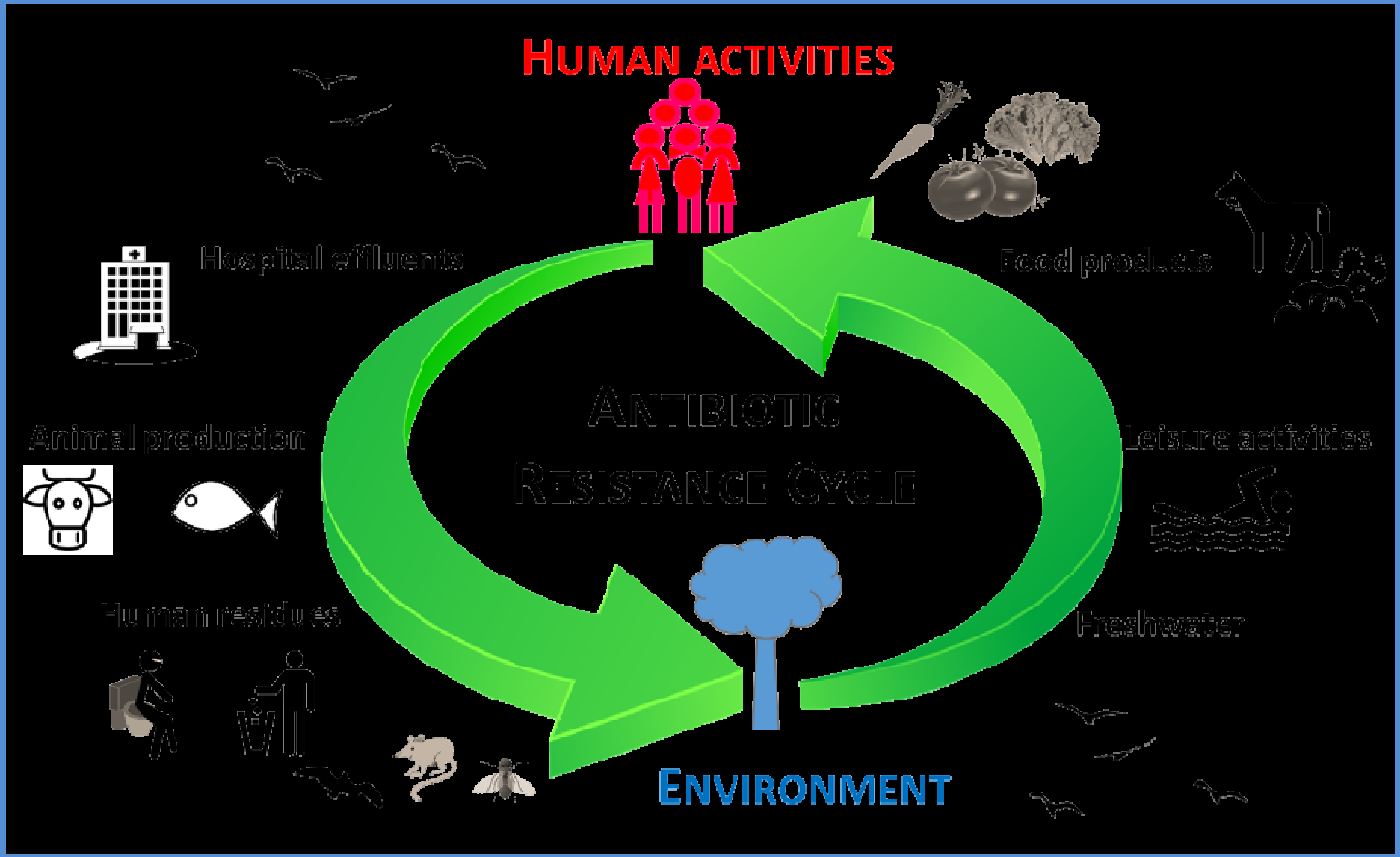
Due to the phenomenon of resistance, the huge advantage gained over the last century will be lost:

1. Infectious diseases	2. Cancer	3. Surgical procedures
• Pneumonia, • Tuberculosis, • HIV • Malaria.	Antibiotics are crucial to help chemotherapy and implicitly to fight with associated infections	• Organ transplantation • Cesarean section, • Prosthesis • Acute abdomen • Tendon surgery

antibiotic resistance is a major problem that encompasses the

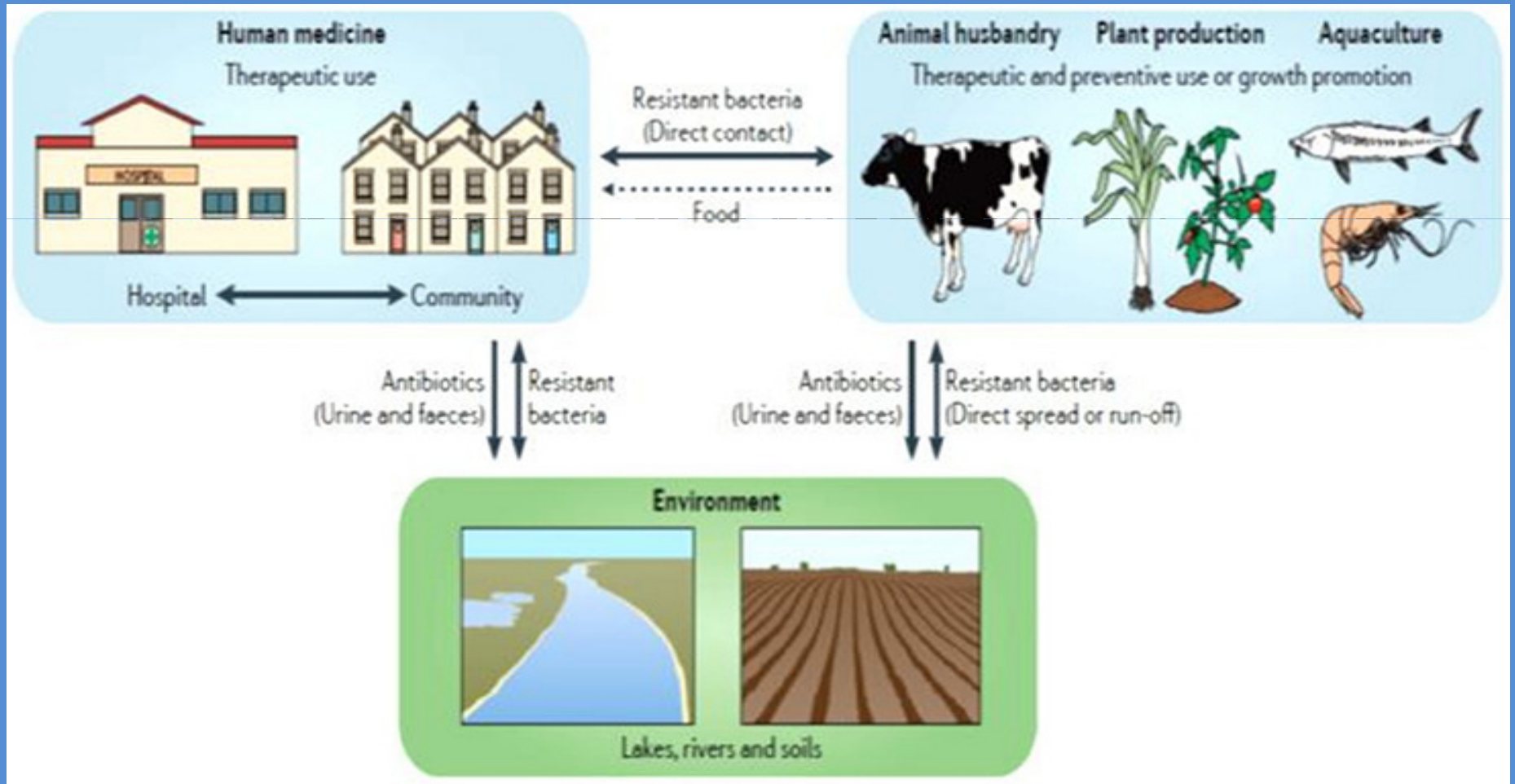


- Antibiotic-resistant bacteria can be contacted by humans
- (i) indirectly along the food chain through the consumption of contaminated food or derived food
- (ii) following direct contact with the colonized / infected animals or through biological substances such as blood, urine, feces, saliva (Changetal., 2015).
- This phenomenon is considered a cross-sectoral problem because antibiotics are commonly used in aquaculture, animal farms and crops, and antibiotic-resistant bacteria and resistance genes can spread to any level of the food chain.

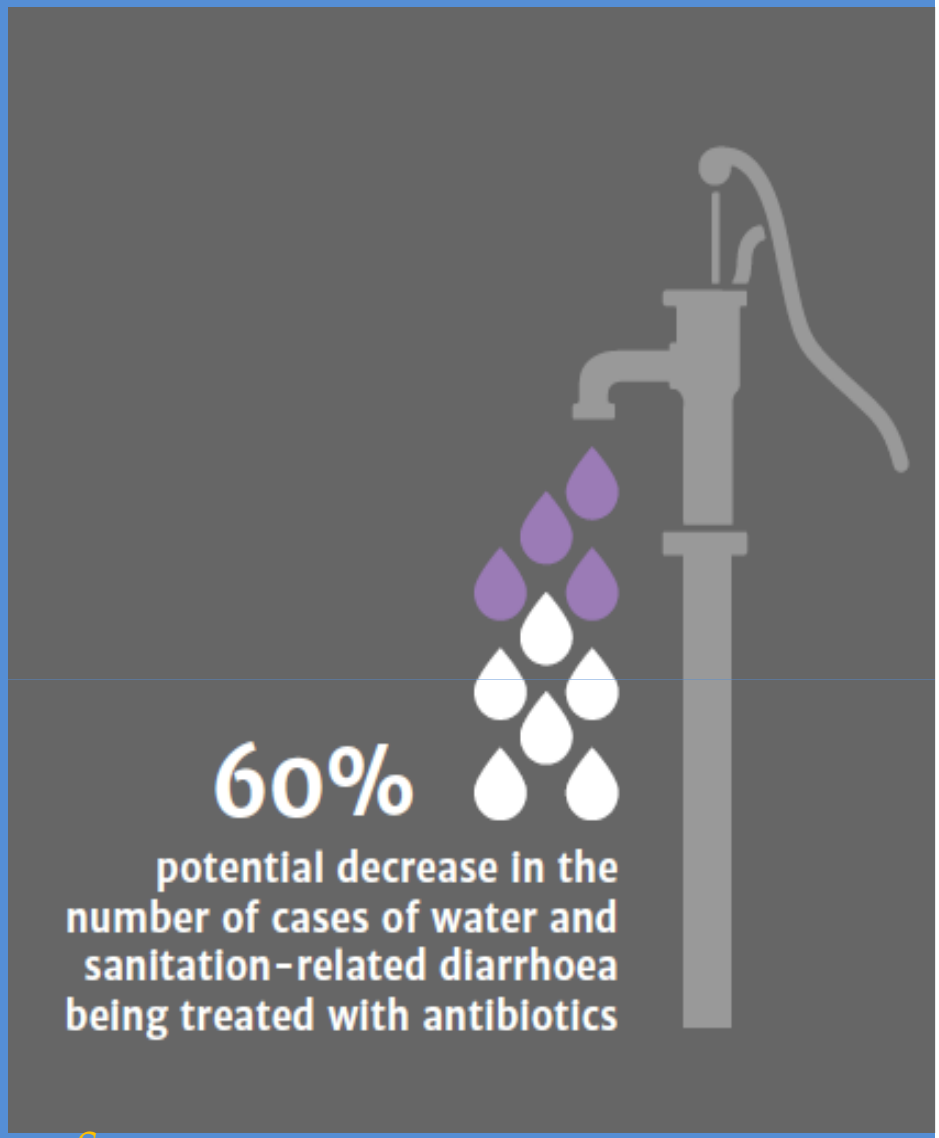

HUMAN ACTIVITIES
Hospital effluents
Food products
ANTIBIOTIC
RESISTANCE CYCLE
Animal production
Leisure activities
Human residues
Freshwater
ENVIRONMENT

- In this regard, several studies and reports have been carried out demonstrating a high resistance to ampicillin, sulfonamides, and tetracyclines for *Salmonella spp*. isolated from humans, *Salmonella* and *Escherichia coli* isolated from piglets for fattening and from calves.
- High proportions of ampicillin, sulfonamides and tetracycline-resistant strains have been identified, while third-generation cephalosporin resistance was less common.
- Thus, in a report from the EU level, the resistance level for *E. coli* isolated from calves under one year old was generally lower than those from pigs for fattening, with a proportion of 43.8% at tetracyclines, 34.4% for sulfamethoxazole, 29% for ampicillin and 24.7% for trimethoprim.
- About piglets, the level of resistance to *E. coli* was 52.1% for tetracycline, 42.4% for sulfamethoxazole, 38.5% for ampicillin and 32.2% for trimethoprim.

- **Antibiotic residues, antibiotic-resistant bacteria, and resistance genes** are considered **to be environmental pollutants and responsible for accelerating antibioresistance.**

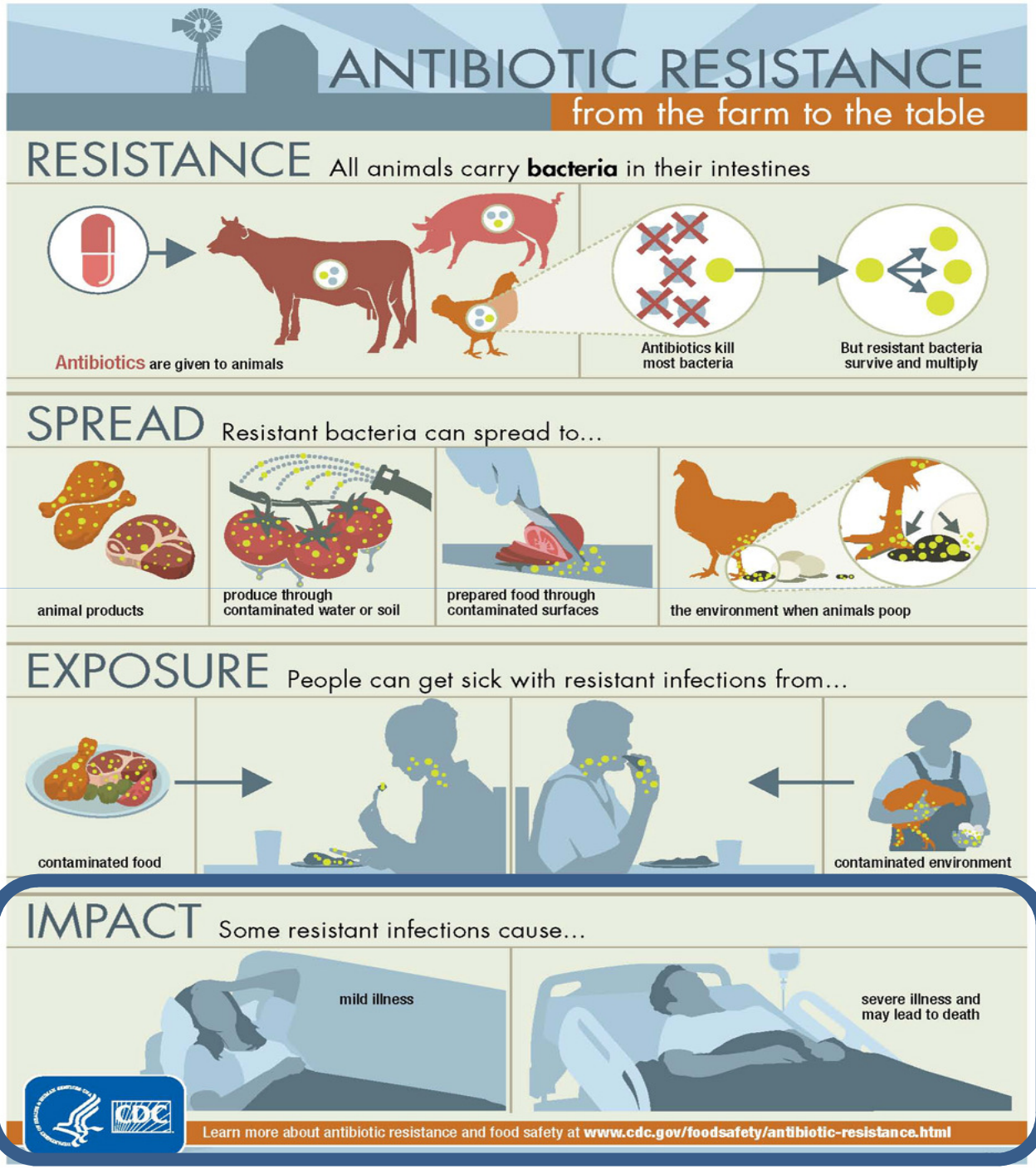


Antibiotics that have been identified in surface waters often included:
macrolides,
sulfonamides
tetracyclines,
chloramphenicol,
chlortetracycline,
sulfamethazine
lincomycin
trimethoprim,
sulfadimethoxin
sulfamethazine.



Source:
https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

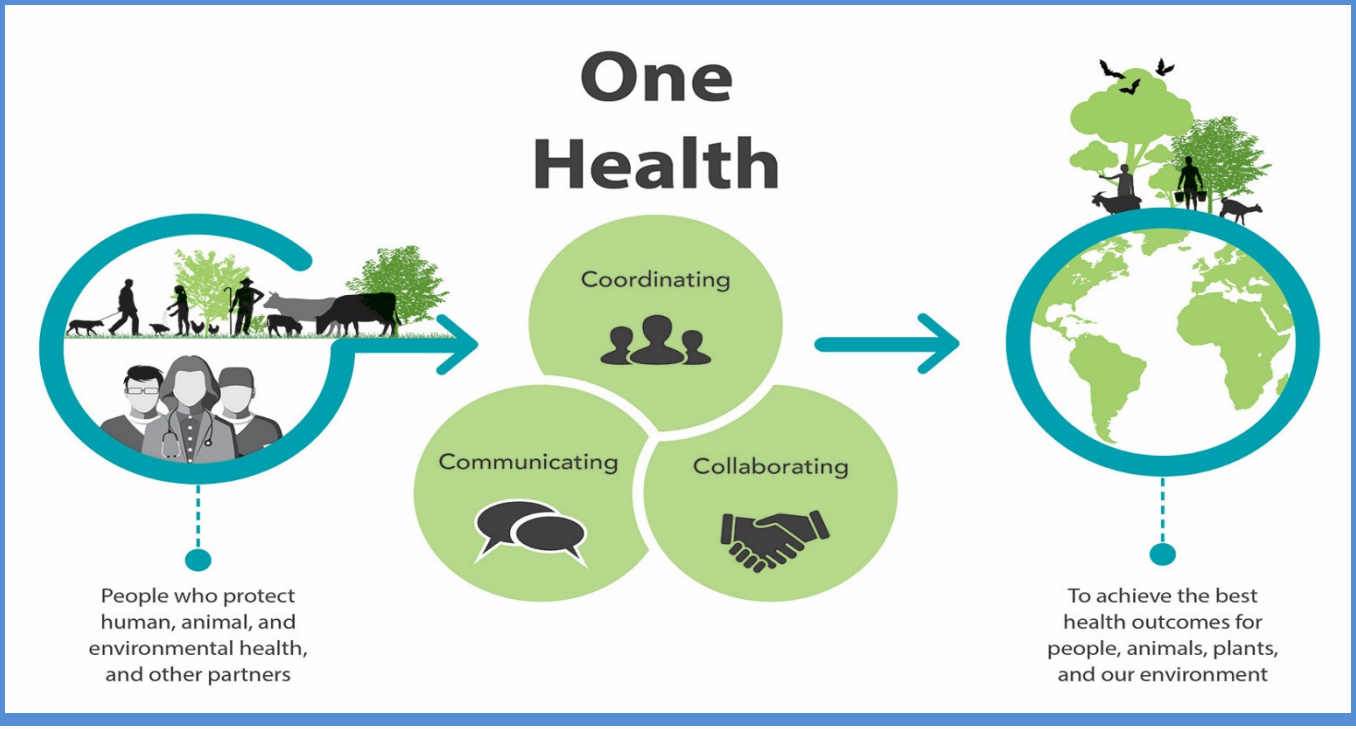
- **AC / AR link demonstrated statistically ($p < 0.05$) for:**
- **fluoroquinolone / E.coli in both sectors**
- **cephalosporins from generation 3 & 4 in E.coli in humans**
- **tetracyclines in E. coli in animals**
- **carbapenems and polymyxins / K. pneumoniae in humans**
- **macrolides in animals have been associated with resistance to Campylobacter spp. in animals and humans.**
- **cephalosporins generation 3 & 4 = resistance to fluoroquinolones / E.coli in humans**
- **fluoroquinolones / Salmonella spp. and Campylobacter spp. in humans has been linked to the consumption of fluoroquinolones in animals**


ANTIBIOTIC RESISTANCE
from the farm to the table
RESISTANCE All animals carry bacteria in their intestines
Antibiotics are given to animals
Antibiotics kill most bacteria
But resistant bacteria survive and multiply
SPREAD Resistant bacteria can spread to...
animal products
produce through contaminated water or soil
prepared food through contaminated surfaces
the environment when animals poop
EXPOSURE People can get sick with resistant infections from...
contaminated food
contaminated environment
IMPACT Some resistant infections cause...
mild illness
severe illness and may lead to death
Learn more about antibiotic resistance and food safety at www.cdc.gov/foodsafety/antibiotic-resistance.html

One World, One Health and "One Resistome"....

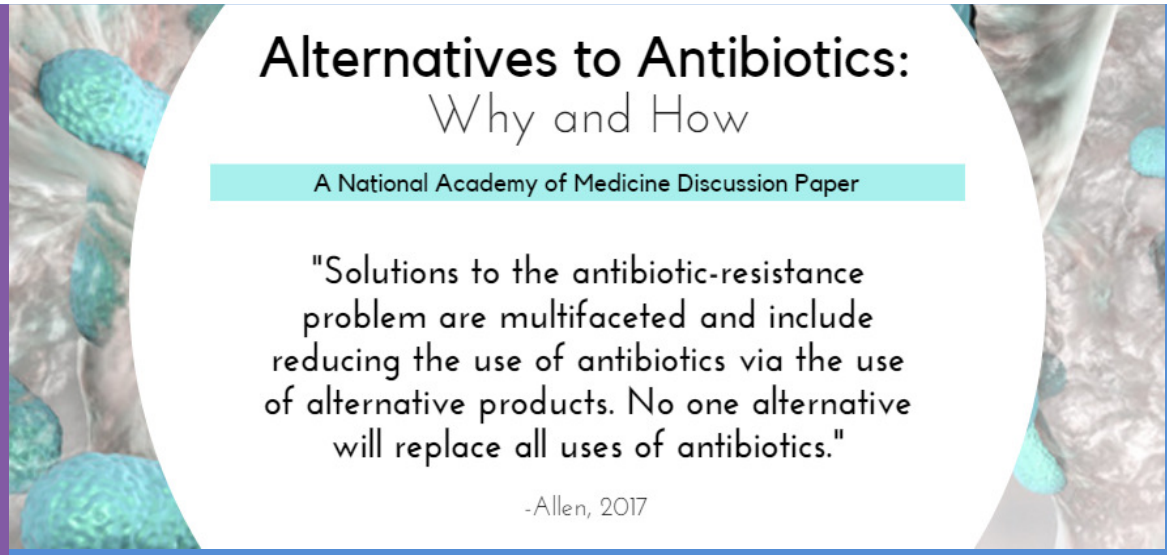
Given that the countries of the G20 account for 80% of total world meat production..., a large part of antibiotic consumption in livestock and the likelihood of generating drug resistance, currently rests with them.

(Jim O'Neil, 2016)



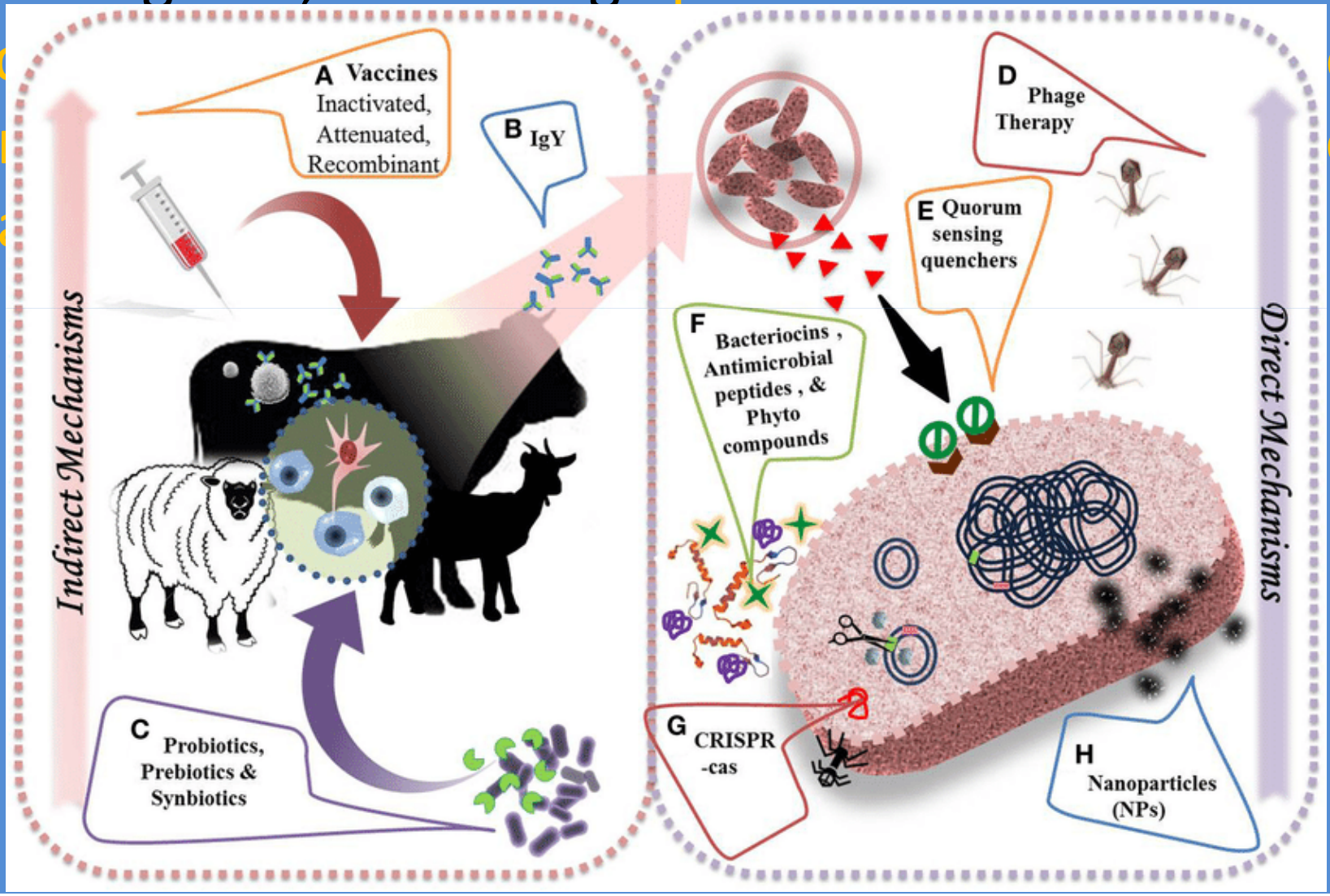
The basics of public health – clean water, good sanitation and hygiene, infection prevention and control and surveillance – are as critical for reducing the impact of antimicrobial resistance as they are for infectious disease control

Dr Keiji Fukuda,
the Director General's Special
Representative for Antimicrobial Resistance
at the World Health Organization (WHO)



ew ideas and strategies against resistance phenomenon to anti-infective structures

- As the resistance to conventional antimicrobials increases, different alternatives to antimicrobials are investigated, including products obtained from ac es, pr es, na



- The fight against AMR is constant, and the discovery of new antimicrobials is critical.
- Although antibiotics have served humanity for the past 70 years, the ability of bacteria to adapt quickly has led to other options being explored.
- To increase the efficacy of antifungals, it is desired to obtain substances with reduced toxicity, increased bioavailability, with an improved antifungal spectrum, to which currently plans to combat resistance are added.
- Elucidation of the mode of action of a potential antifungal compound can shorten the time until the ideal drug is obtained.
- The small antifungal molecules obtained from natural products can represent structural models for the study of the structure-activity relationship, thus offering more information for potential new antifungal agents.

Limitations of today's drugs:

- unsatisfactory bioavailability
- limited effect
- potentially high cytotoxicity
- long and frequent treatments

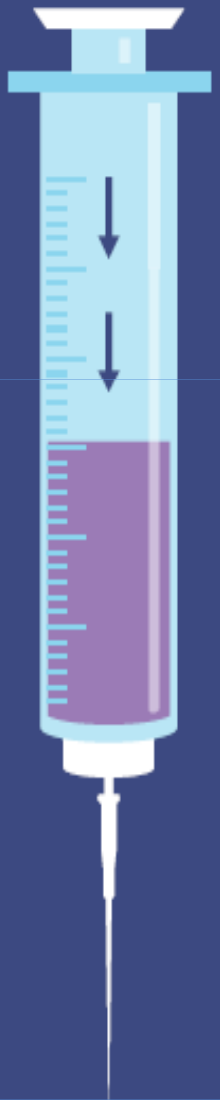
In this context, specific characteristics are needed that can reduce morbidity and mortality!

Tackling antimicrobial resistance requires a wide range of approaches and developing alternatives to antibiotics, in humans and animals, is critical to the fight.

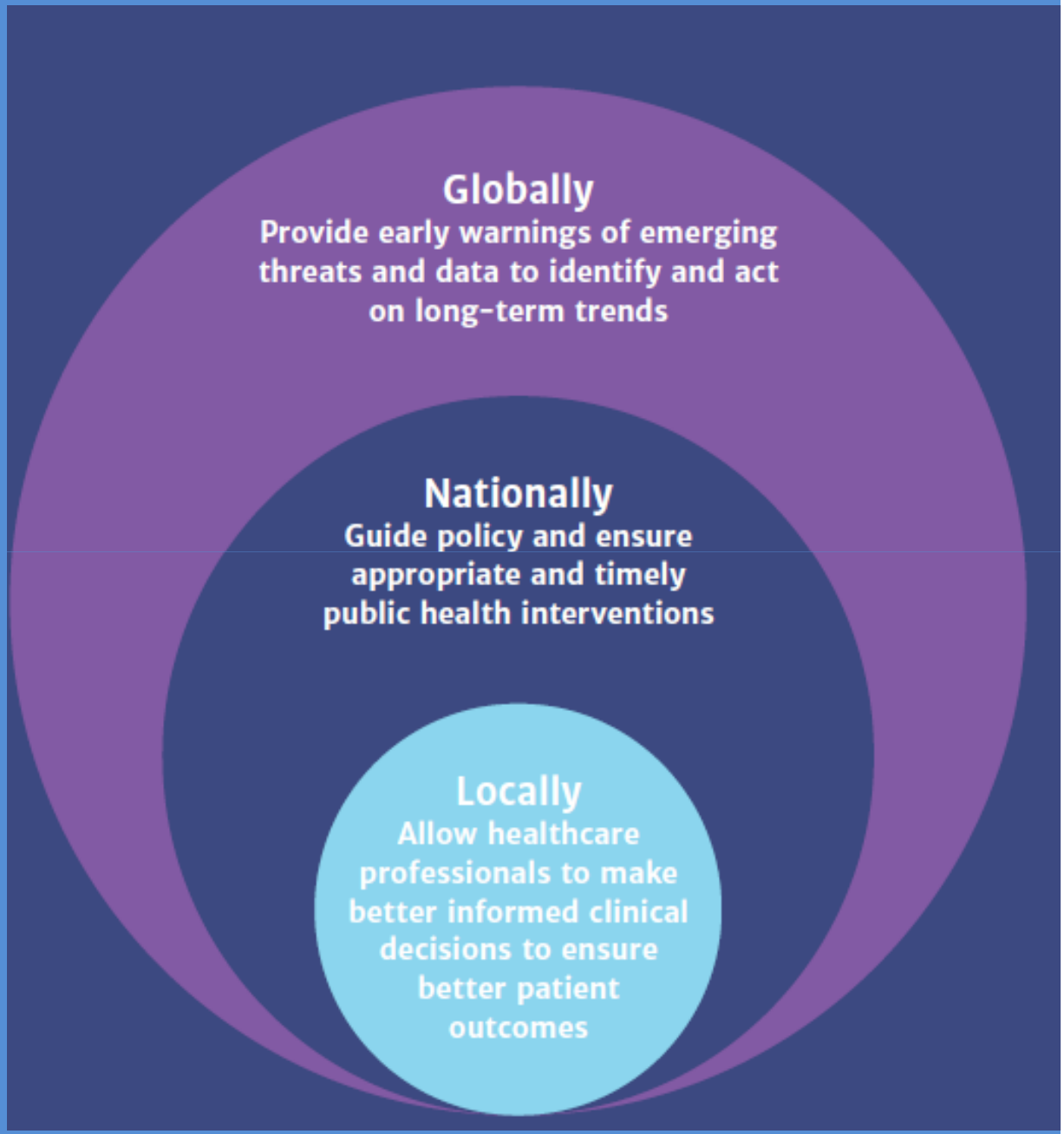
Vaccines have a vital role to play in combating drug resistance, by preventing infections in the first place

Sally Davies,
Chief Medical Officer for England

Universal coverage by a pneumococcal conjugate vaccine could potentially avert 11.4 million days of antibiotic use per year in children younger than five, roughly a 47% reduction in the amount of antibiotics used for pneumonia cases caused by *S. pneumoniae*.



47%
reduction in
antibiotic use



Source: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Attractive alternatives to vaccines could be:

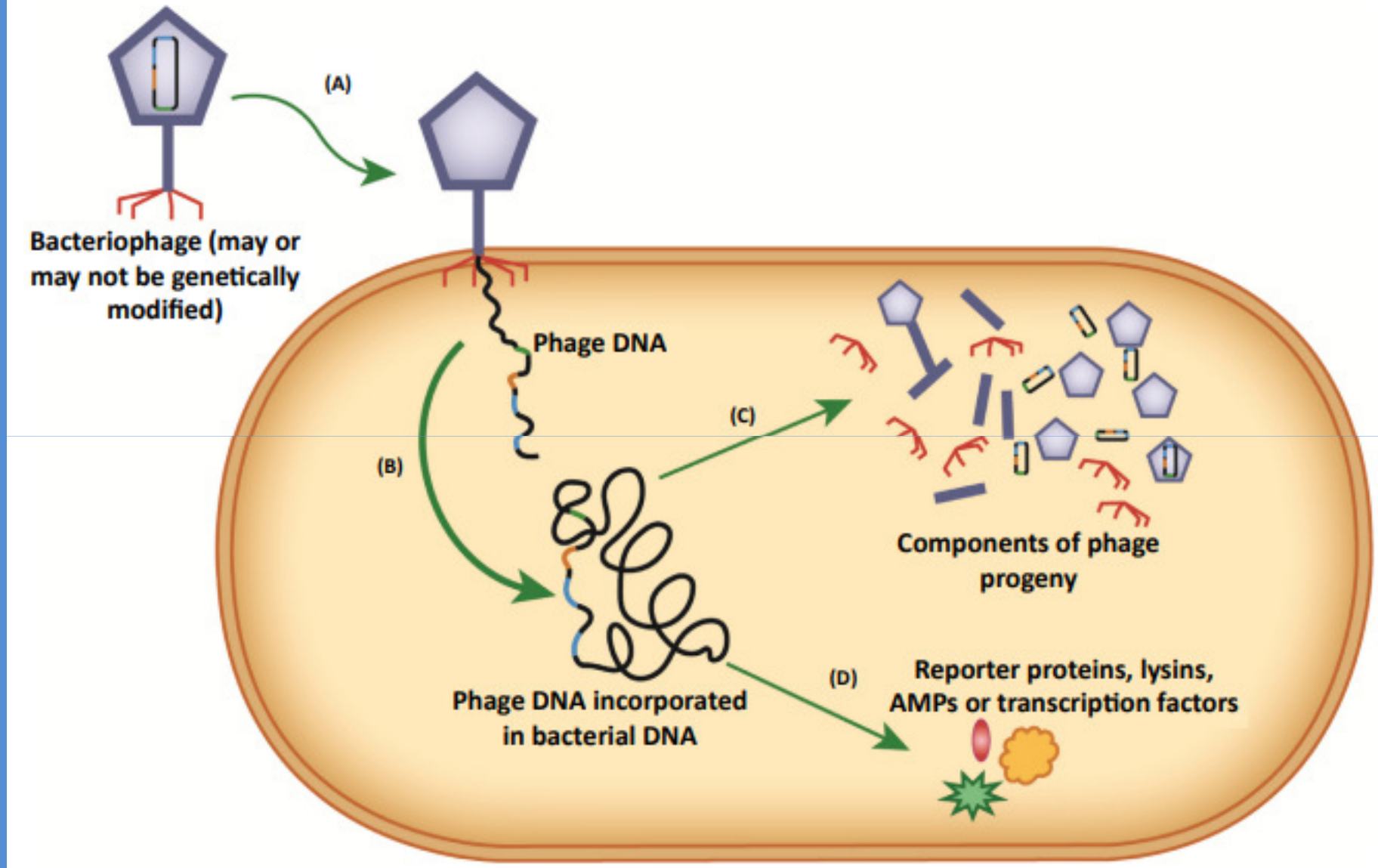
- **specific pathogenic antibodies**
- **immunomodulatory agents**
- **Bacteriophages**
- **antimicrobial peptides**
- **pro-, pre- or symbiotic products**

Examples of reducing antibiotic use:

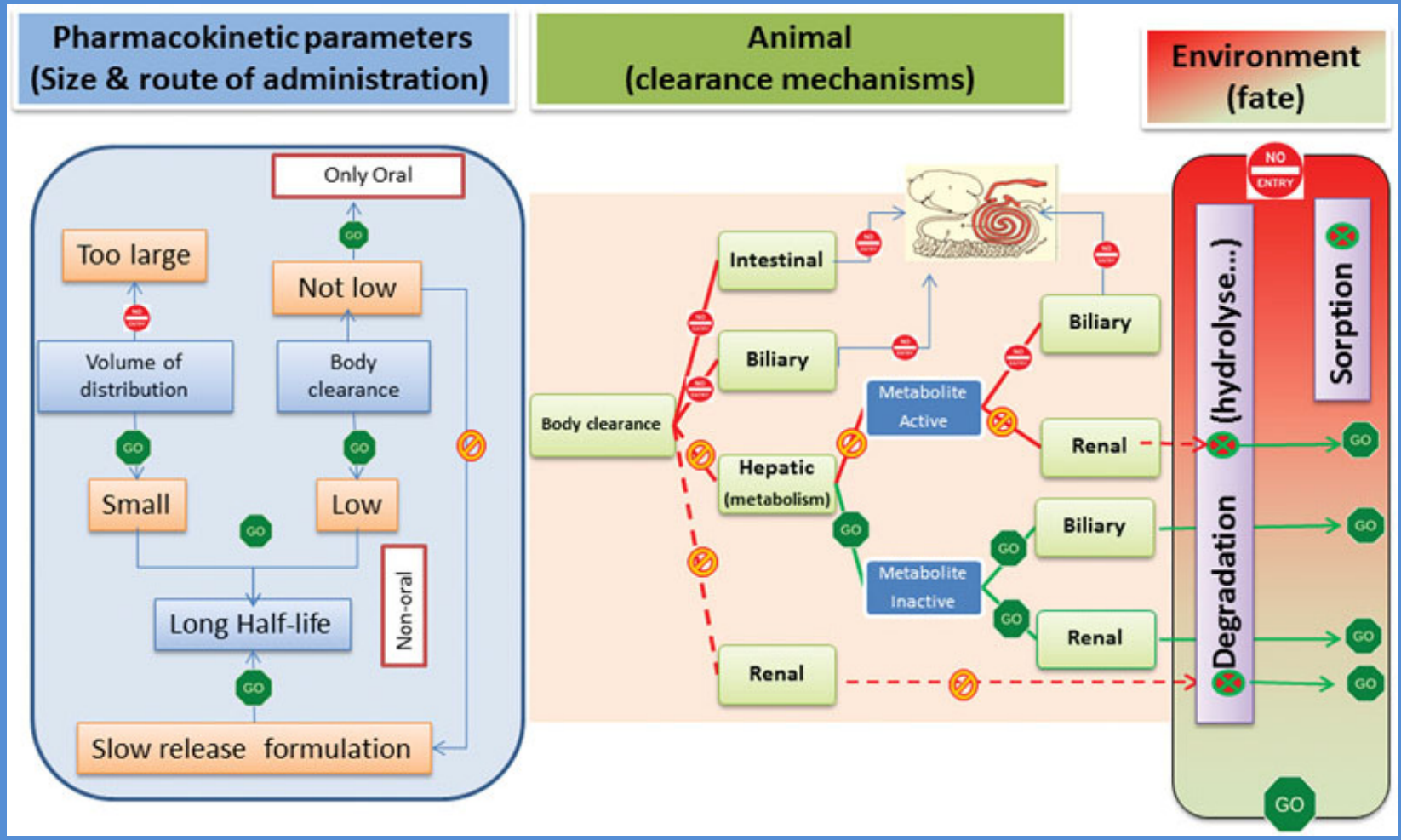
- an effective vaccine for the prevention of furunculosis in Norway (Midtlyng et al., 2011).
- use of a vaccine to prevent enteritis produced by Lawsonia intracellularis in pigs.

Bacteriophages

- Bacteriophages or viruses that "eat" bacteria have been used to treat infected animals before conventional antibiotics are used for the same purpose.
- They attach to the surface of the bacterial cell and then inject the genetic material into the bacterial cytoplasm.
- It then takes control of the host cells, which results in the synthesis of phage components and the assembly of new phages in infected bacteria.
- Bacterial lysis occurs and the release of phage offspring can begin a second cycle of infection.
- Bacteriophages - an alternative but selective approach to target pathogenic bacteria and "ignore" commensal bacteria

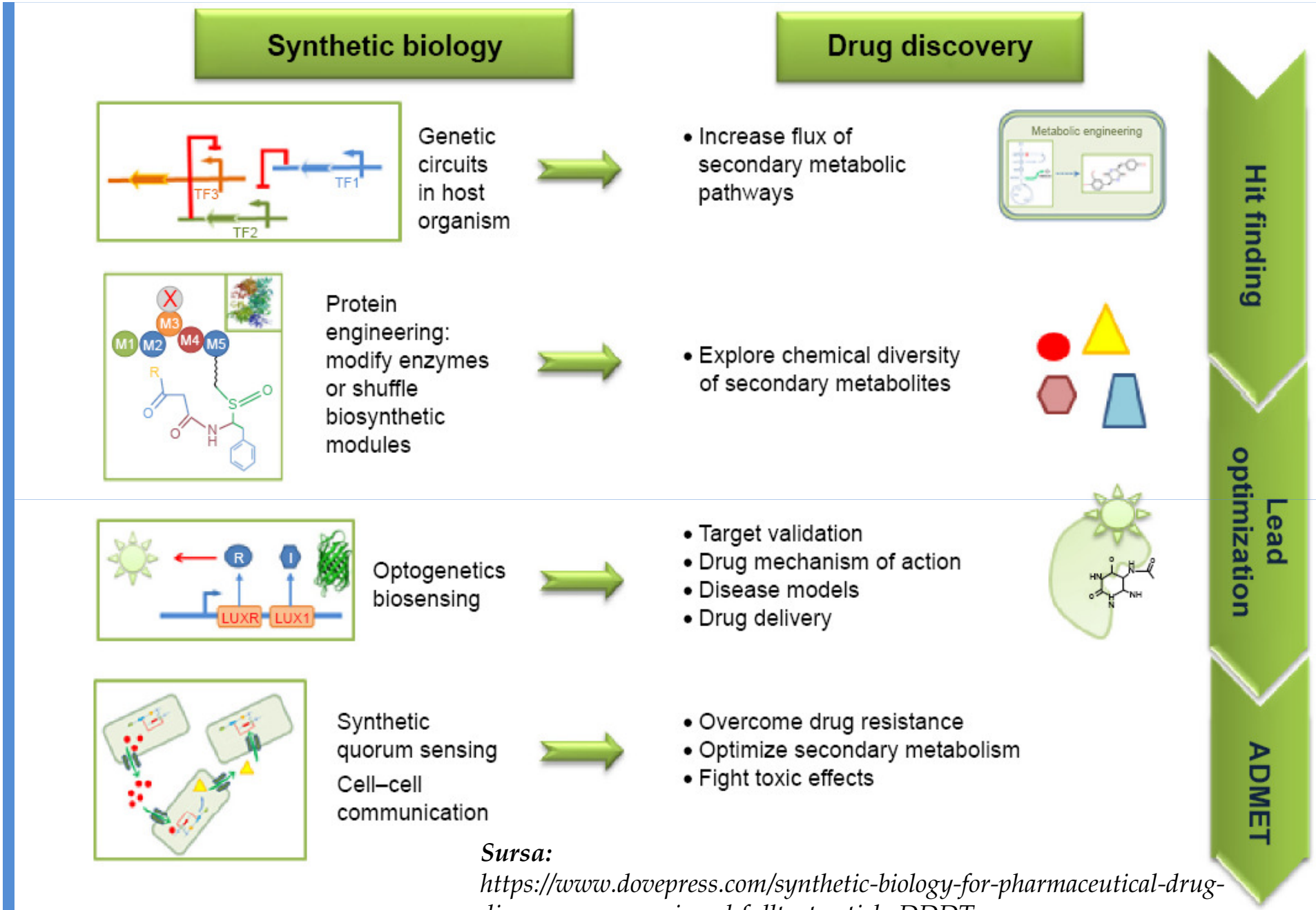

(A)
Bacteriophage (may or may not be genetically modified)
Phage DNA
(B)
(C)
Components of phage progeny
Phage DNA incorporated in bacterial DNA
(D)
Reporter proteins, lysins, AMPs or transcription factors

Eco-green medicines …



Lipophilic drugs are selected in V.M. for the following reasons:

- the need for retardation and correlation with T 1/2.
- development of new potent and eco-green pharmacons: ceftaroline, telavancin, and dalbavancin.



Sursa:
https://www.dovepress.com/synthetic-biology-for-pharmaceutical-drug-discovery-peer-reviewed-fulltext-article-DDDT

Figure 2 Synthetic biology tools in various steps of drug discovery.
Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; TF, transcription factor.

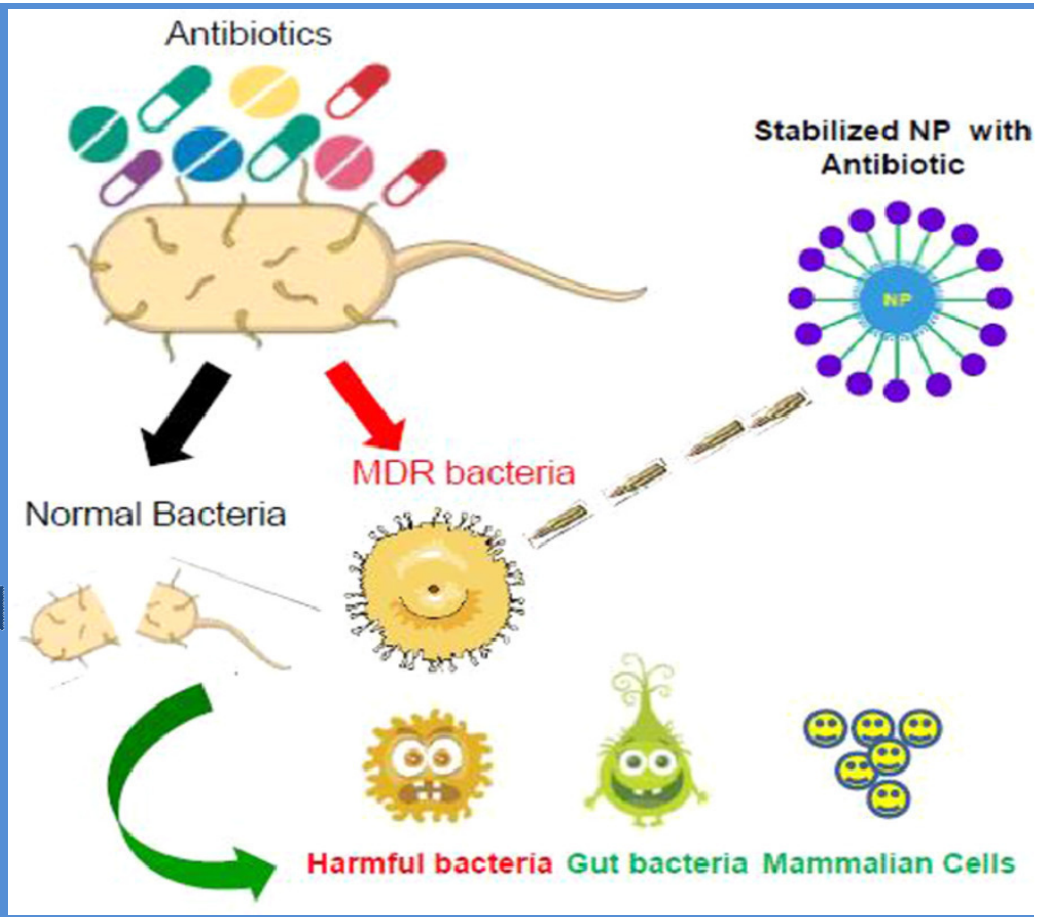
Other viable alternatives

Nanotechnology

a) minimal invasive therapy

b) high density functions

c) concentrations in very small volumes



Nano Devices

- maximizes therapeutic activity
- minimizes toxic side effects
- they target specific cells and not tissues

Silver nanoparticles - conjugation with different antifungal substances

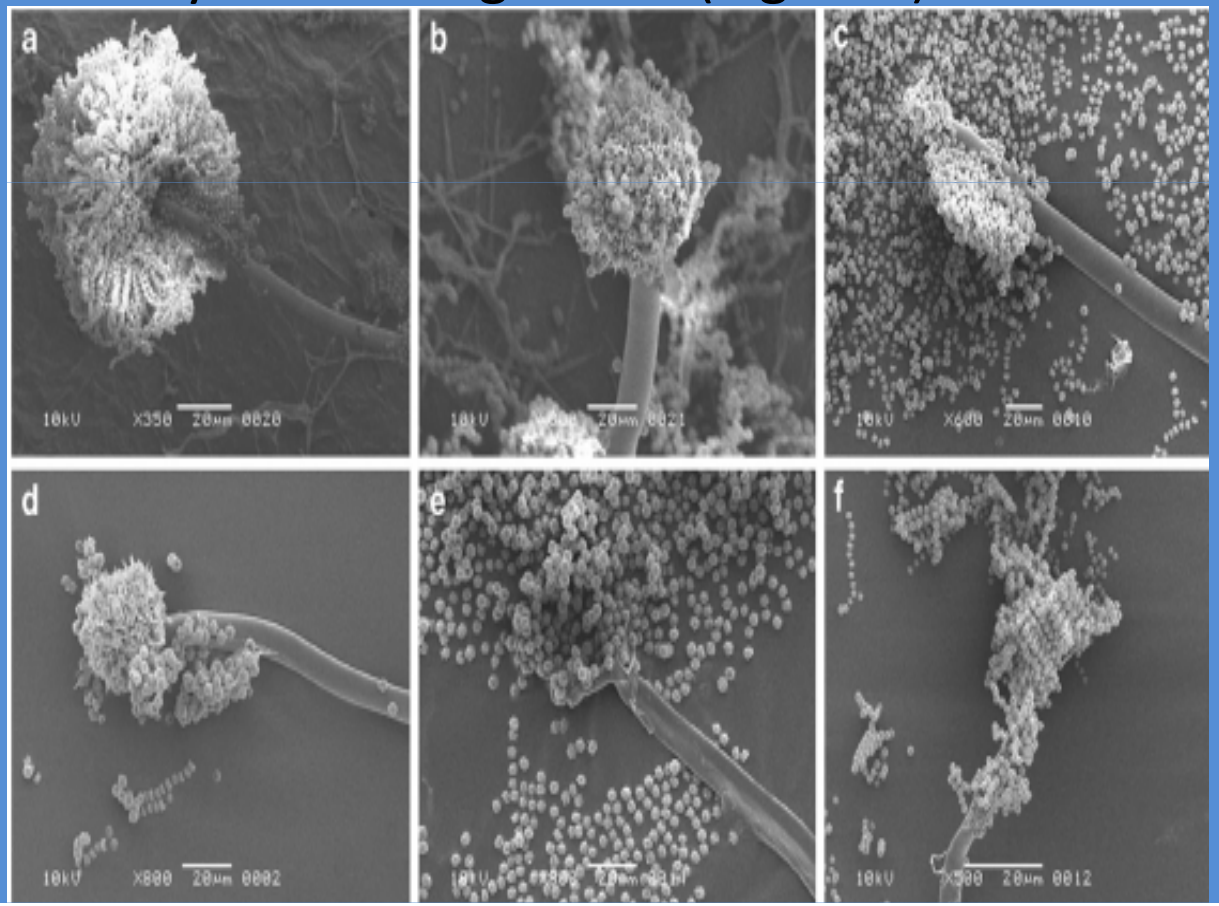
- **Silver nanoparticles (SA)** have recently been developed as a new class of antimicrobial agents against numerous pathogenic microorganisms
- **Nanoparticles - efficient drug carriers -** associated with increasing drug bioavailability and minimizing the inhibition of the action of some substances

- **Silver nanoparticles - in single therapy -** have a broad spectrum of antimicrobial activity, including: ***Candida albicans, C. tropicalis, C. parapsilosis, C. glabrata, Trichophyton rubrum, Trichosporon asahii, Aspergillus niger, Rhizoctonia solani, Curvularia lunata, Colletotrichum spp. And Fusarium spp.***

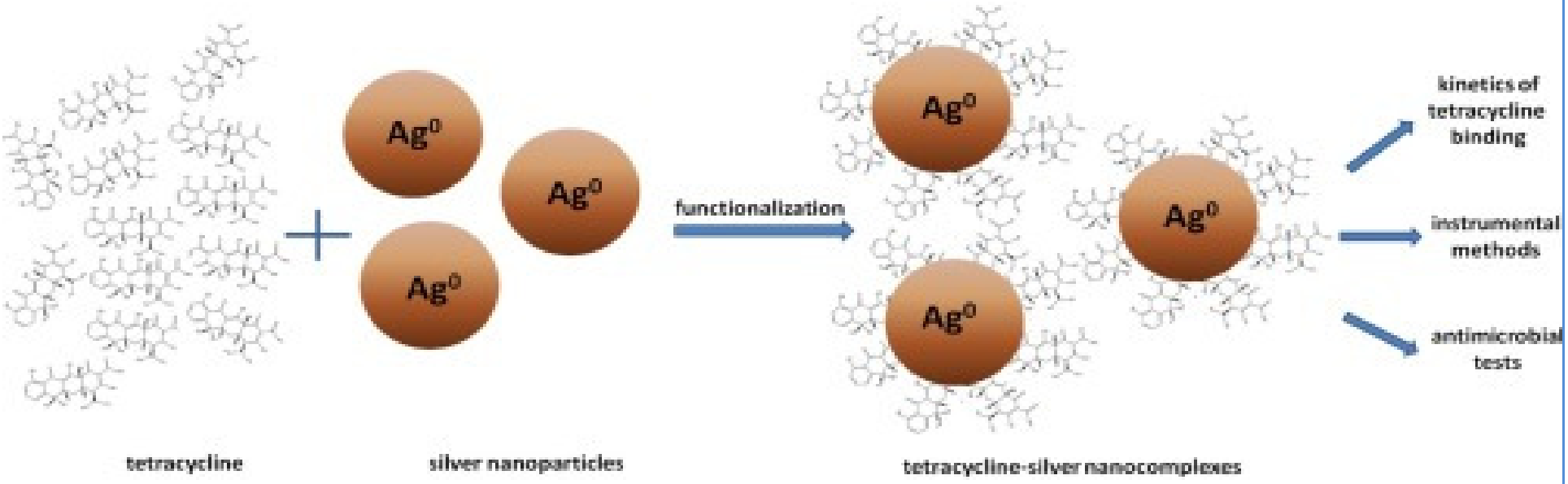
- Silver nanoparticles conjugated to nystatin and flucitosine:

- - the fungistatic effects on *Candida albicans* of nystatin and flucitosine are only 30%, respectively 33%, compared with 50%, respectively 62% when the two antifungal substances were conjugated with SA, at the same concentration. These data indicate that a conjugation with SA enhances the antifungal potency of different substances on *C. albicans*

- In the treatment of *A. brasiliensis* with nystatin and nystatin-SA, a slight to severe deformation was observed, respectively an irregular surface (Figure b-c), in the treatment with flucytosine, it underwent changes compared to the control (Figure d). The flucytosine-SA complex resulted in a severe morphological change (Figure e), and the combination of nystatin-SA and flucytosine-SA in 1: 1 mixture completely destroyed the fungal cells (Figure f)



The silver nanoparticles, in addition to the conjugation with different active substances, are incorporated into clothing, footwear, paints, wound dressings, various devices, cosmetics and plastics, due to their antibacterial properties.

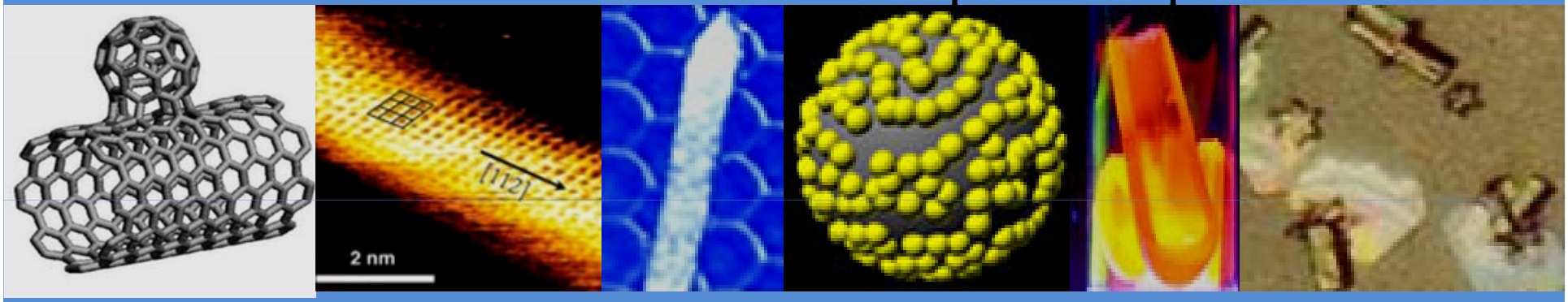


	Ag-CGG	Ag-CGG-tetracycline
Pseudomonas aeruginosa	10 ± 2	13 ± 2
Escherichia coli	9 ± 2	11 ± 2
Klebsiella pneumoniae	10 ± 1	12 ± 1
Staphylococcus aureus	7 ± 0	11 ± 1
Staphylococcus epidermidis	12 ± 0	15 ± 0

Inhibition zones (mm) caused by silver nanoparticles CGG 11n functionalized and not functionalized with tetracycline

Nano-devices

Nanotube Nanowires Nanostructures Nanospheres Nanopores Fullerenes

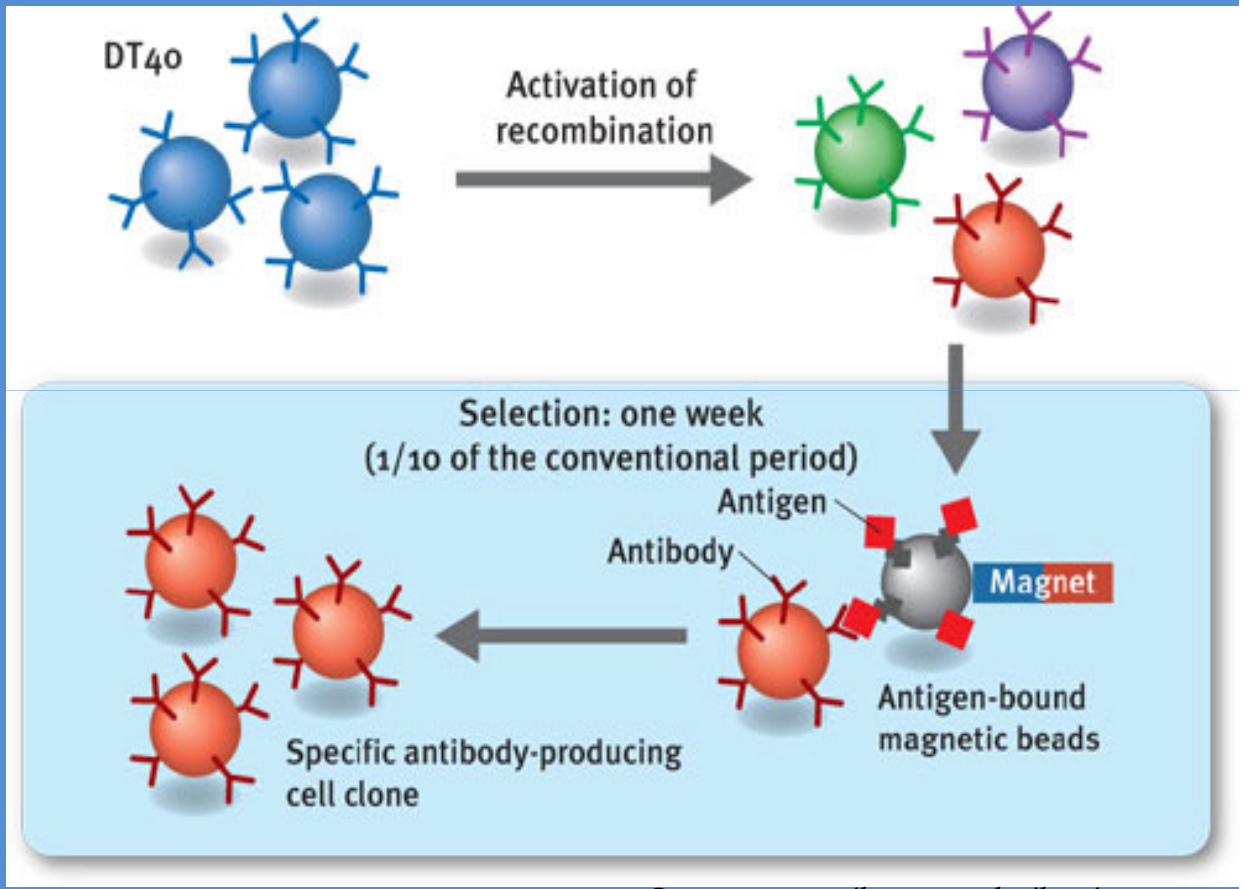


increasingly sophisticated due to molecular studies
this fact allowing them to:

1. target,
2. identify,
3. precisely release the active substance,
4. monitor the therapeutic effectiveness in real time.

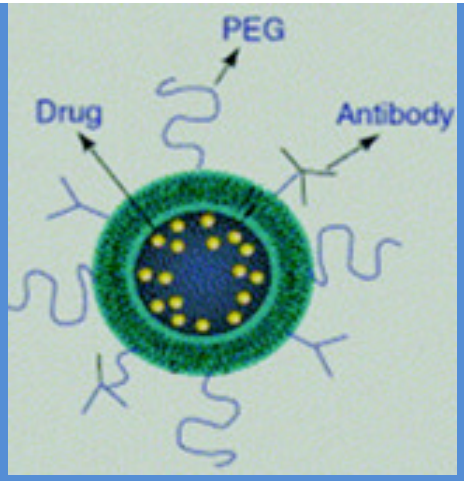
ADLib

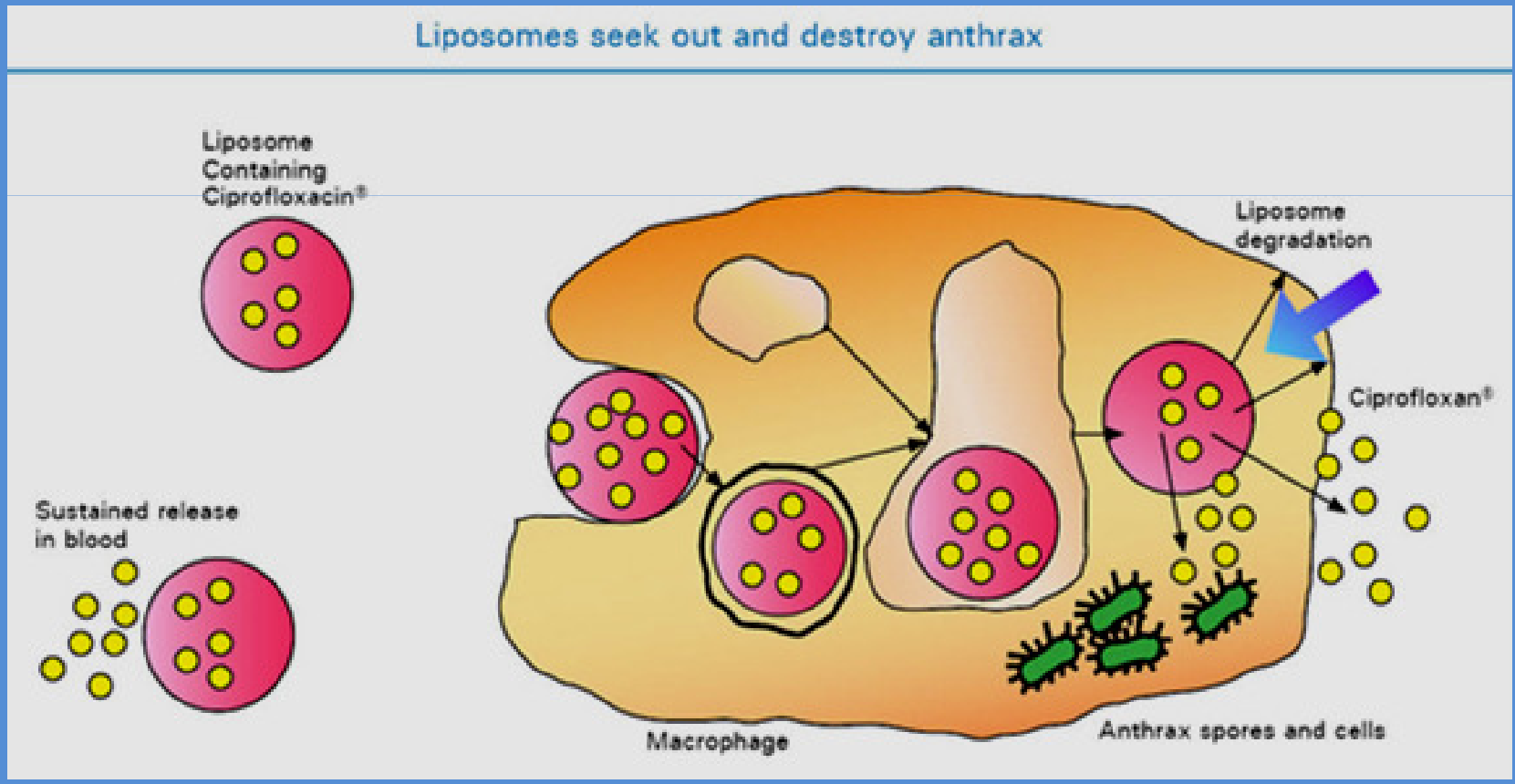
New way to produce antibodies! It opens the way of applications in the field of diagnostic products for personalized treatments.



Source: *www.rikenresearch.riken.jpg*

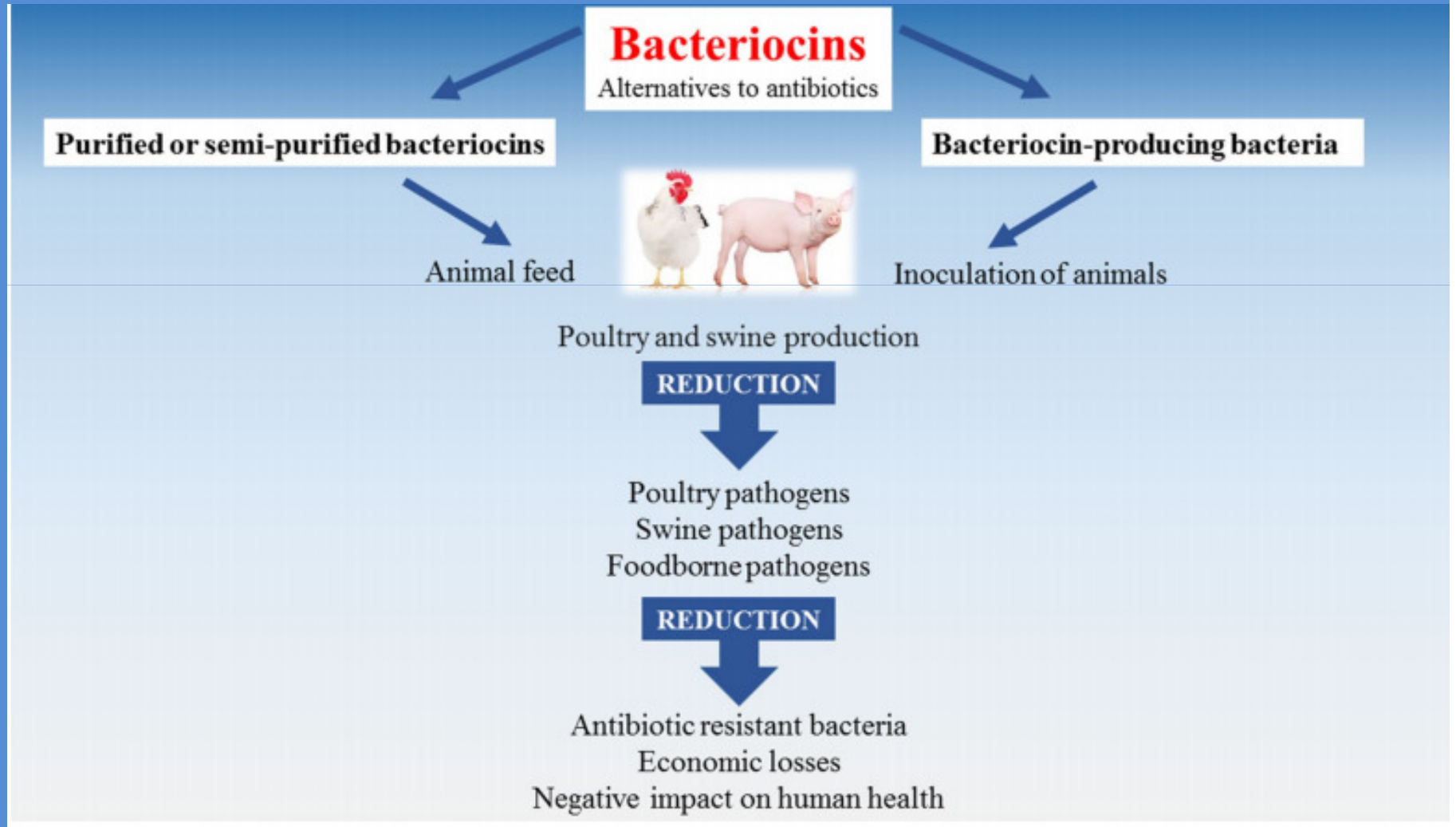
Liposomes



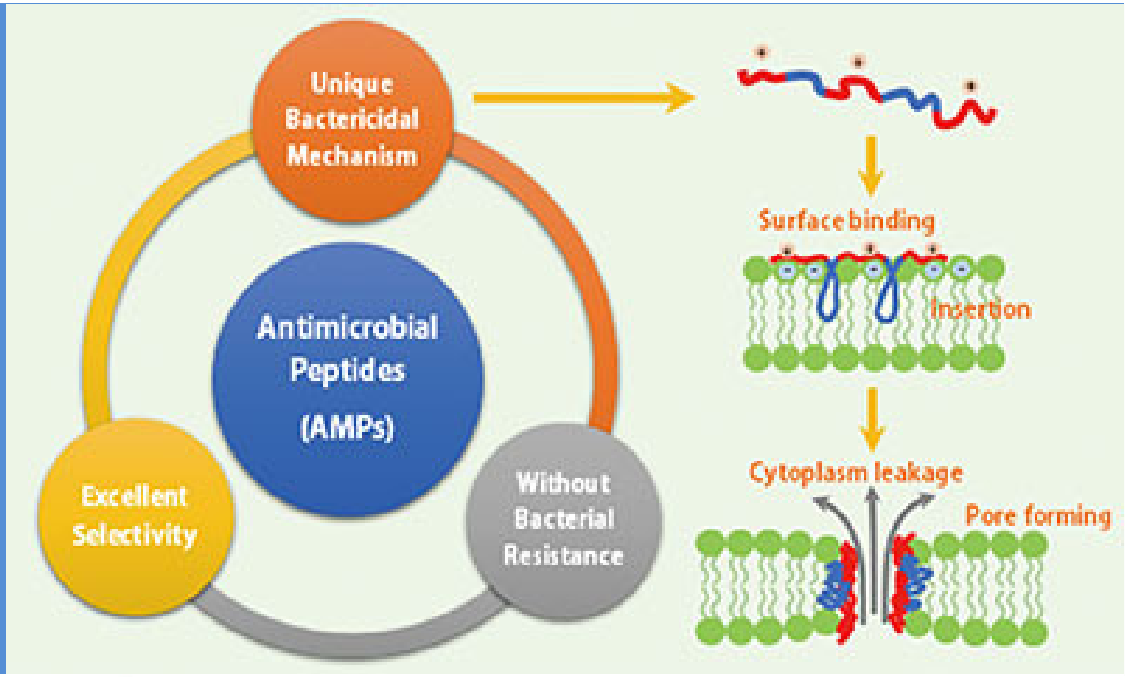


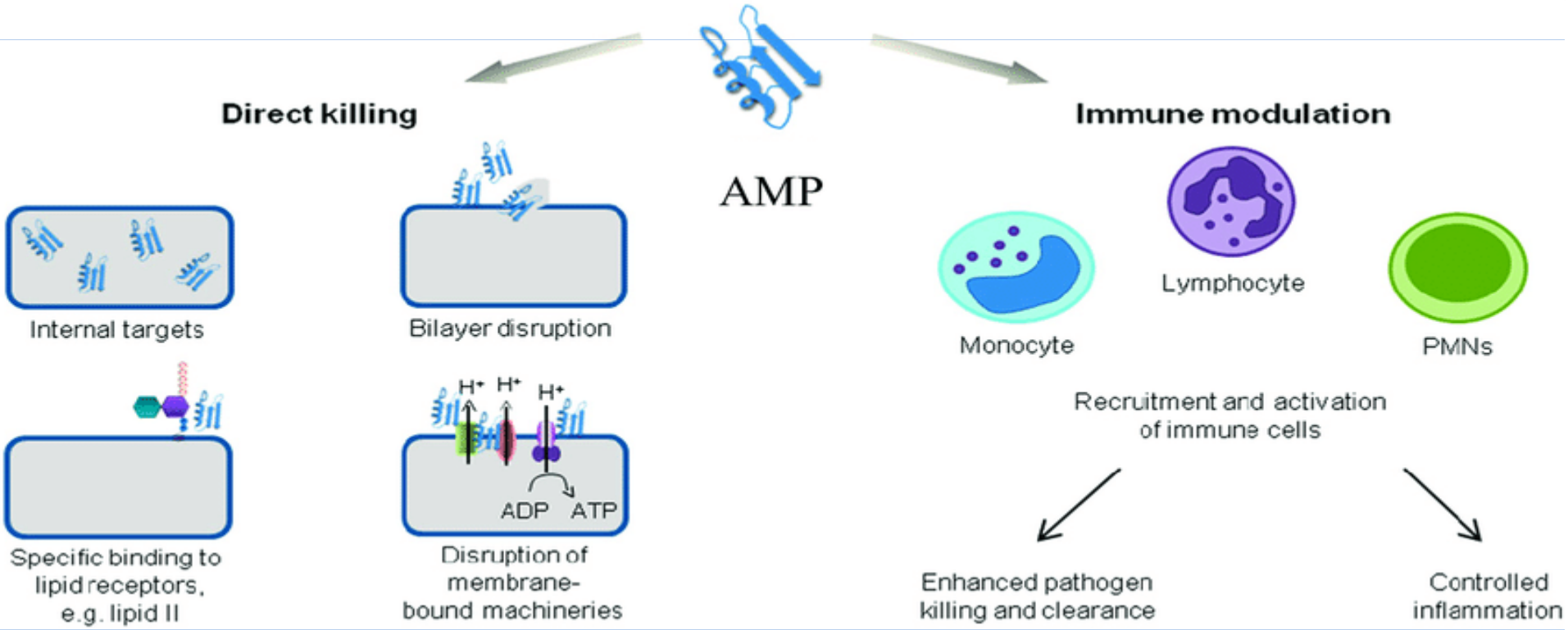
***Source**: http://s3.amazonaws.com/readers/healthmad/2007/07/22/43701_0.jpg*

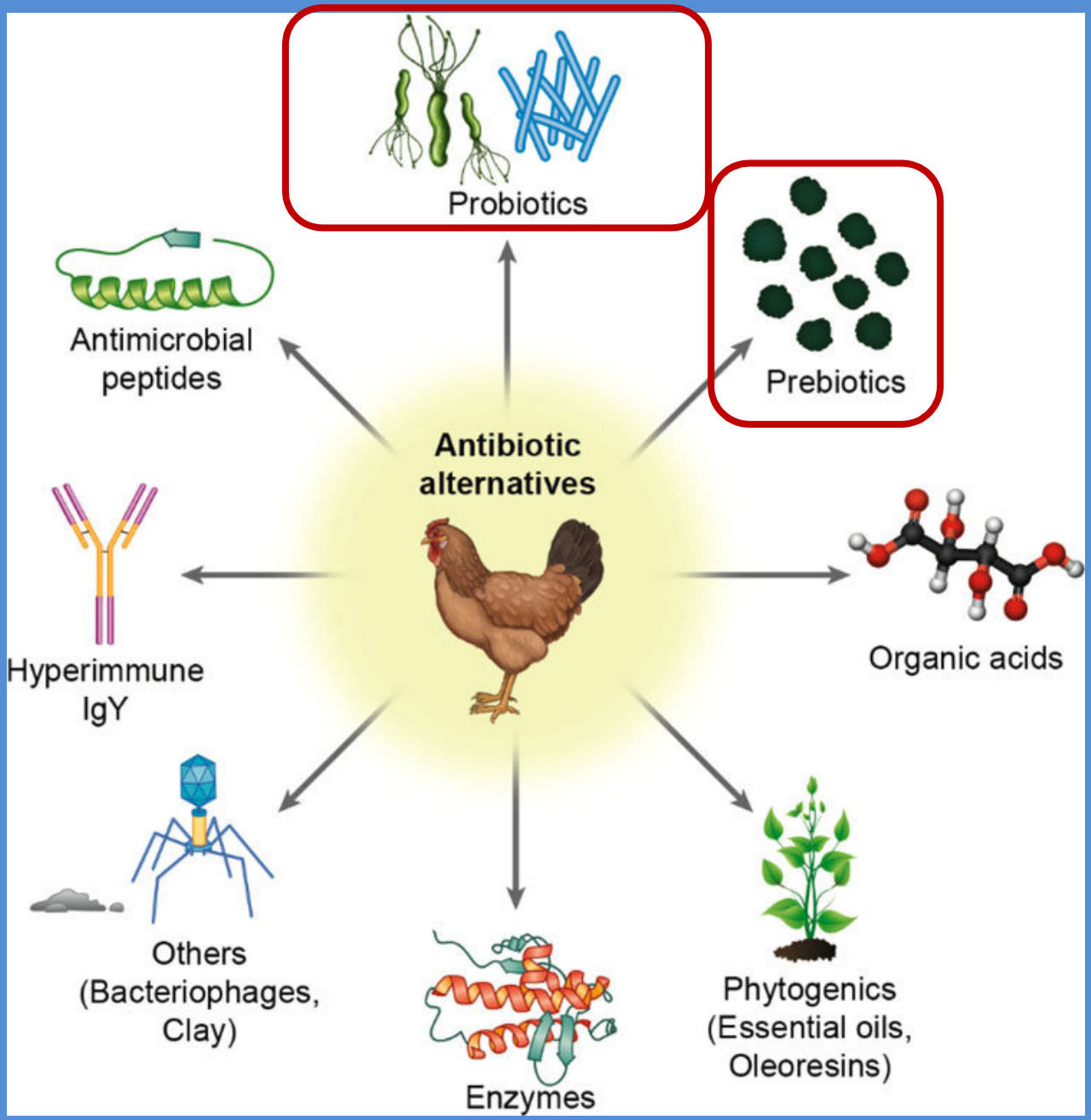
Bacteriocins



Antimicrobial peptides





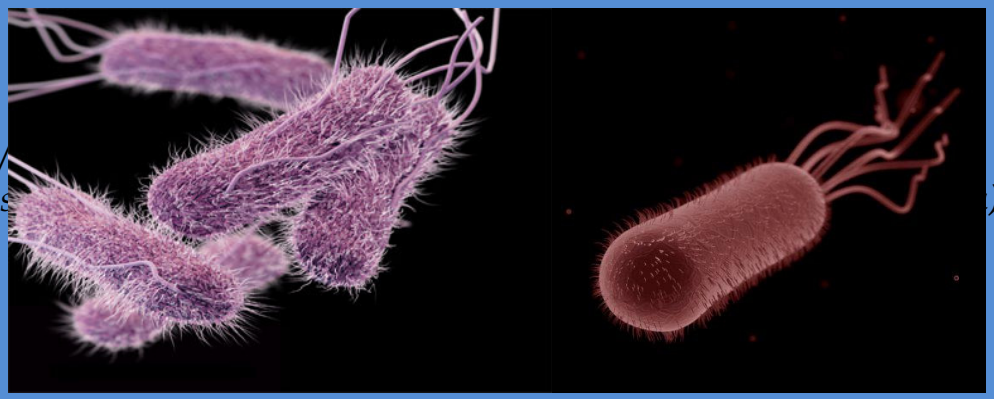

Probiotics
Prebiotics
Antimicrobial peptides
Antibiotic alternatives
Hyperimmune IgY
Organic acids
Others (Bacteriophages, Clay)
Enzymes
Phytogenics (Essential oils, Oleoresins)

Genetics

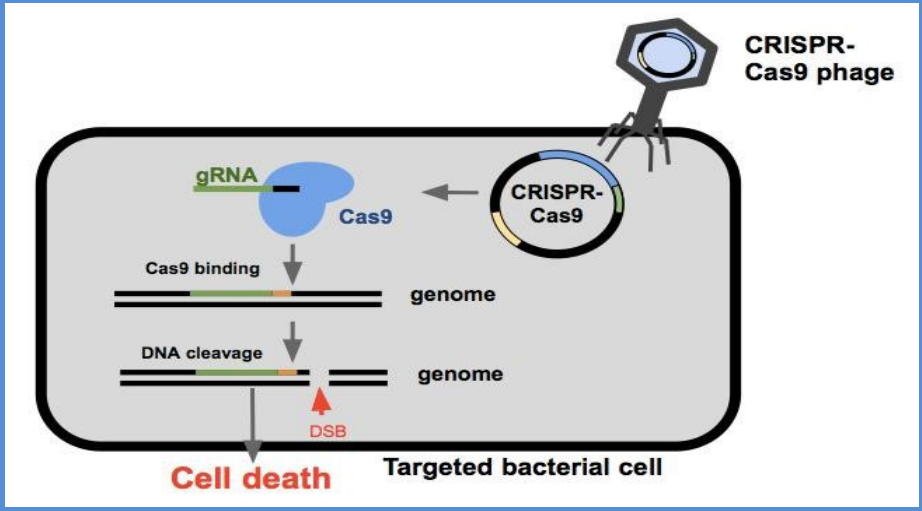
The researchers genetically reprogrammed an *E. coli* serotype. It can kill even other groups of bacteria responsible for hard-to-treat infections present in: lungs, bladder or implanted medical devices.

Genetic reprogramming of E. coli

Source: http://sa-mcp.s3.amazonaws.com/up-image/
http://www.biosciencetechnology.com/sites/bios



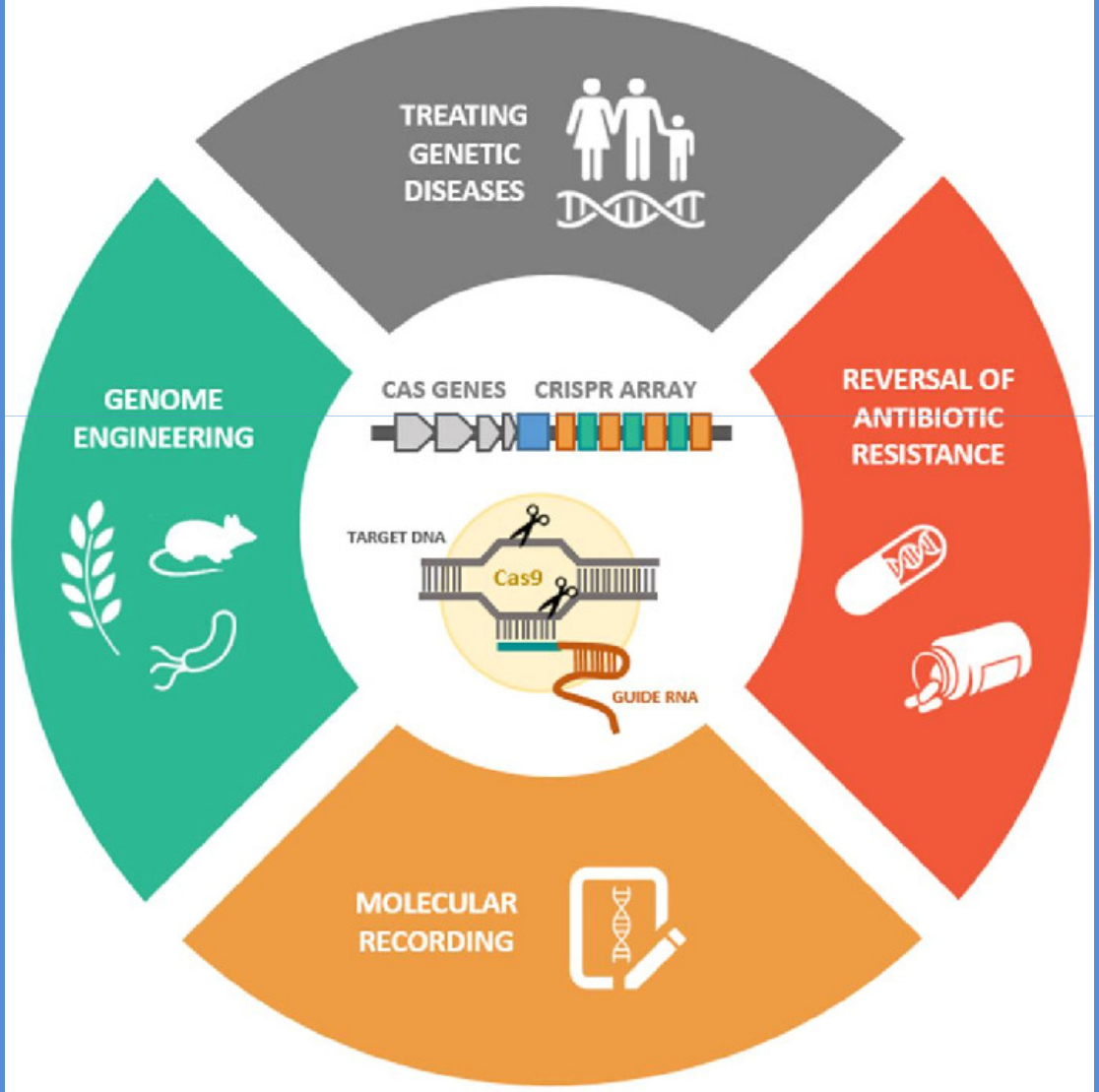
CRISPR-Cas9



- The researchers designed a plasmid that can eliminate an antibiotic resistance gene from *Enterococcus faecalis* bacteria, which could lead to new methods to combat antibiotic resistance.
- The mechanism used to eliminate antibiotic resistance genes is the specialized protein, CRISPR-Cas9, which can "cut" almost anywhere in the DNA.
- Along with CRISPR-Cas9, DNA homologous RNA sequences from the antibiotic resistance gene were added to the designed plasmid.
- These RNAs guide CRISPR-Cas9 to make the reductions in the right places.

CRISPR-Cas system

Key elements and applications



The main alternatives - their advantages and disadvantages

	Advantages	Disadvantages
Bacteriophages	• Self-replicating pharmaceuticals • Selective for specific strains of bacteria • According to genetic engineering	• immunogenicity • pharmacokinetics • Release of bacterial endotoxins • Development of resistance
Lysine	• According to genetic engineering • Selective for specific strains of bacteria • It is not prone to developing resistance	• production • Insufficient data
CRISPR/Cas9	• Can be adjusted for a variety of antimicrobial applications • Decreased antibiotic use • Specificity for pathogenic strains	• Large-scale production is expensive • Toxicity
Antimicrobial peptides	• It is not prone to developing resistance • Broad spectrum activity	• Large-scale production is expensive • Sensitive to proteolysis • Toxicity
Bacteriocins	• Specificity for pathogenic strains of bacteria • Resistance to heat and UV	• Large-scale production is expensive • Sensitive to proteolysis
SMAMPsa (Synthetic antimicrobial peptides)	• Easy to synthesize • It is not prone to developing resistance • Broad spectrum activity	• Toxicity • The route of administration
Probiotics	• Easy availability	• Used especially for intestinal infections
Antibodies	• Selective for specific strains of bacteria • Protected microflora	• High cost of production • Period of validity

- **Plants** are a primary source for obtaining new medicinal products (Hostettman, 1999).
- **The active principles of plants** have various **pharmacodynamic actions,** such as **anti-inflammatory, anti-carcinogenic and anti-atherosclerotic, antibacterial, antifungal, antiviral, antimutagenic and anti-allergic** (Ikken et al., 1999; Noguchi et al., 1999; Mishra et al., 2009)

Plants and natural products with antifungal activity

- The use of herbs in the treatment of skin conditions, including fungal infections, is an old practice in many parts of the world.
- This use was supported by the isolation of the active antifungal compounds from the plant extracts
- It is known that the essential oils of many plants have both antibacterial and antifungal activity
- *Caryophyllus aromaticus* (the tree that produces the famous "cloves") is famous for its eugenol-rich volatile oil with certain antibacterial and antifungal properties for the treatment of dermatophytosis in animals.

- Another species recognized for its antimicrobial properties is *Malaleuca alternifolia*, which produces a volatile oil (tea tree oil) rich in **terpineol, terpine and cineole**, with a wide range of bacterial and fungal action.
- gel with 5% volatile oil of *Malaleuca alternifolia* **(Tea Tree Anti-Fungal Gel)** for the topical treatment of dermatophytosis

- **Garlic (Allium sativum)**

exhibit antibacterial and antifungal activity, thus essential oils, extracts in water and ethanol inhibit the *in vitro* growth of species:

Bacillus spp., Staphylococcus aureus, Escherichia coli, Pasteurella multocida, Proteus, Pseudomonas aeruginosa, Candida spp. and Cryptococcoccus spp.

- **Hassan and Hany's** study demonstrated the **antifungal activity of Aloe vera gel on dermatophytes**, respectively *Tricophyton verrucosum*
- by daily topical applications, the results being compared with the effects of applying a 10% iodine ointment for 20 days.
- After 6 days of treatment with Aloe vera gel, the activity of the dermatophytes was significantly reduced.
- The lesions gradually began to subside, and within two weeks, the hair began to grow.
- In the iodine-treated group, the lesions receded after ten days of treatment and the hair regression began after two weeks.
- Within a month there was a complete recovery in both treated groups

Plants	Fungal
Kaempferia galanga (flavored ginger)	*Saprolignea parasitica (fish)*
Salvia texana	*Aspergillus fumigatus, Histoplasma capsulatum, Coccidioides immitis, Candida albicans*
Nigella sativa	*Dermatofiți*
Melaleuca alternifolia (tea tree)	*Aspergillus spp., Dermatophytes Candida albicans*
Daucus carota (carrot)	*Cryptococcus neoformans, Dermatophytes*
Anacardium occidentale (the cashew tree)	*Cryptococcus neoformans*
Caryophyllus aromaticus	*Dermatophytes Candidosis*
Centella asiatica	*Candida albicans, Aspergillus flavus, Aspergillus niger*
Aloe vera	*Trichophyton verrucosum*
Hypericum perforatum (Rattles)	*Dermatophytes , Malassezia furfur, Candida albicans*
Mentha spicata	*T.rubrum M. gypseum*
Rosmarinus officinalis	*Microsporum canis*
Artemisia sieberi	*T. rubrum, T. verrucosum, T. schoenleinii, T. mentagrophytes,*

Propolis

- The main components: resins and conditioners (50 - 55%), wax (25 - 35%), aromatic and ethereal oils (10%), pollen (5%) and organic and mineral substances (5%)
- Biological activity: directly related to the content of phenols and flavonoids, it is widely used in the pharmaceutical, cosmetic, veterinary and food industries.
- In addition to antibacterial, antiviral, cytotoxic, immunomodulatory and anesthetic actions, propolis also exhibits important antifungal properties, especially about Candida and dermatophyte species, although it also inhibits the development of other fungal species at relatively low concentrations.
- It promotes antifungal drugs, acting synergistically with them.
- But the extracts 5–10% in propylene glycol, show a certain antifungal effect, even without the association with other antifungals

Plants with antibacterial activity

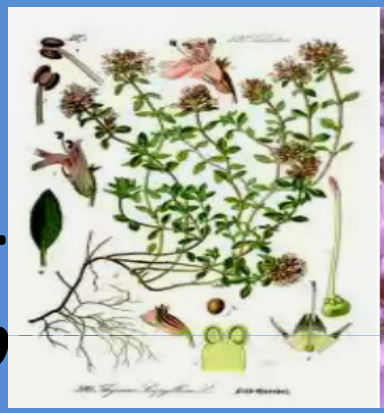
- **Angelica** (Angelica archang

- **Artichokes**(Cynara scolym
  cardunculus L.)

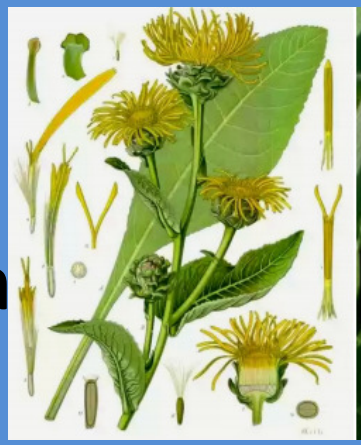
- Arnica (Arnica montana L.)

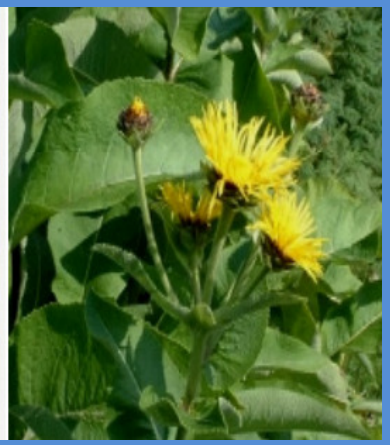
- Thyme (Thymus serpyllus L.

the antibiotic of the p



- Elecampane (Inula helenium





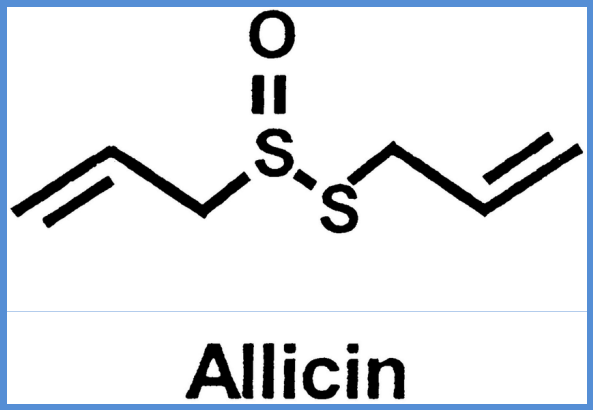
- **Blessed Thistle** (Cnicus benedic

- **Horsetail** (Equisetum arvense L

- **Nettle** (Urtica dioica L., Urtica u

- **Cloves** (Syzygium aromaticum)

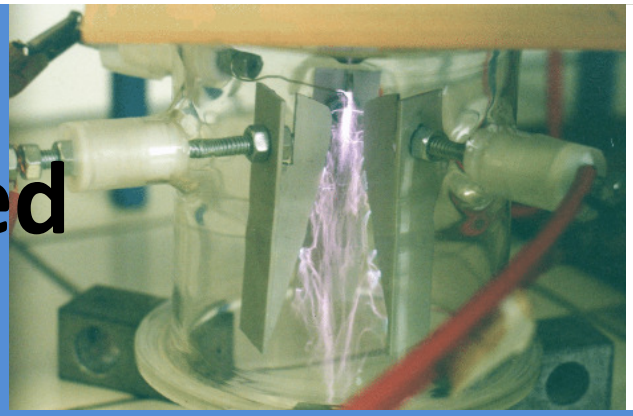
- **Garlic** (allicin)

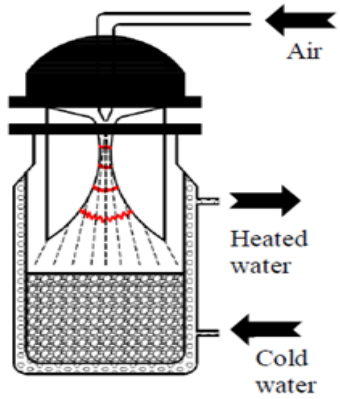


Allicin

- **Salvia**
- **Rosemary**
- **Oregano**

Non-Thermal Plasma-Activated Water (PAW)





❖**Non-Thermal Plasma-Activated Water** is part of a new generation of antimicrobials, extremely effective against microorganisms of medical interest;

Non-thermal plasma can be produced by different types of electric laboratory discharges and fall into the category of advanced oxidation processes.

❖**Glid Arc Tehnology- advantages like:**

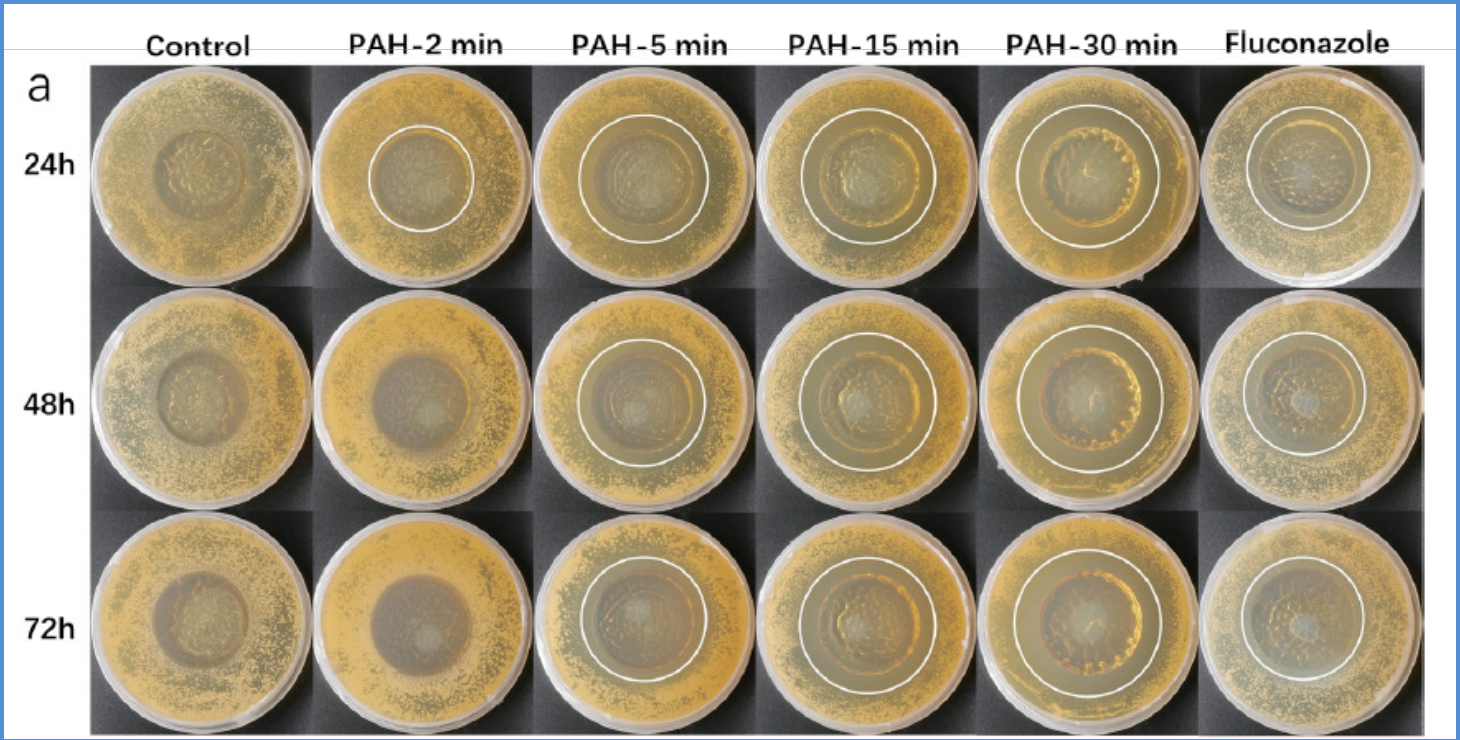
the activation time (20 min) is much shorter than other technologies

activation efficiency is much better

its practical implementation is relatively easy

In a study: A formulation incorporating PAW in the form of a hydrogel (based on acrylamide) was tried and the efficacy of this non-thermal plasma-activated hydrogel was attempted, and the possibility of its use as a new product with antifungal activity.

- The results revealed that the antifungal abilities of PAH-15 min and PAH-30 min were better than that of the fluconazole group.
- This suggests that PAH has better inhibition capabilities than some traditional antifungal agents



PAW+PAH

The groups present in the reactor plasma, mainly the radicals · OH and NO (nitrogen oxides) · when the humid air is the working gas, are precursors of H_2O_2, HNO_3 (nitric acid) and HNO_2 (nitric acid) in water.

a temporary acidification effect of the activated solution, reaching a pH <3.5,

hydrogen peroxide (H_2O_2), nitrate (NO_3) and nitrate (NO_2) groups, responsible for bacterial wall degradation, by oxidizing effect on proteins, lipids and DNA

The duration of chemical activity of these compounds is up to 48 hours by means of controlled diffusion secondary reactions that they produce and therefore the treatment with activated water is efficient even after the interruption of plasma production, so without additional energy consumption, phenomenon called TPDR (post-discharge reactions over time).

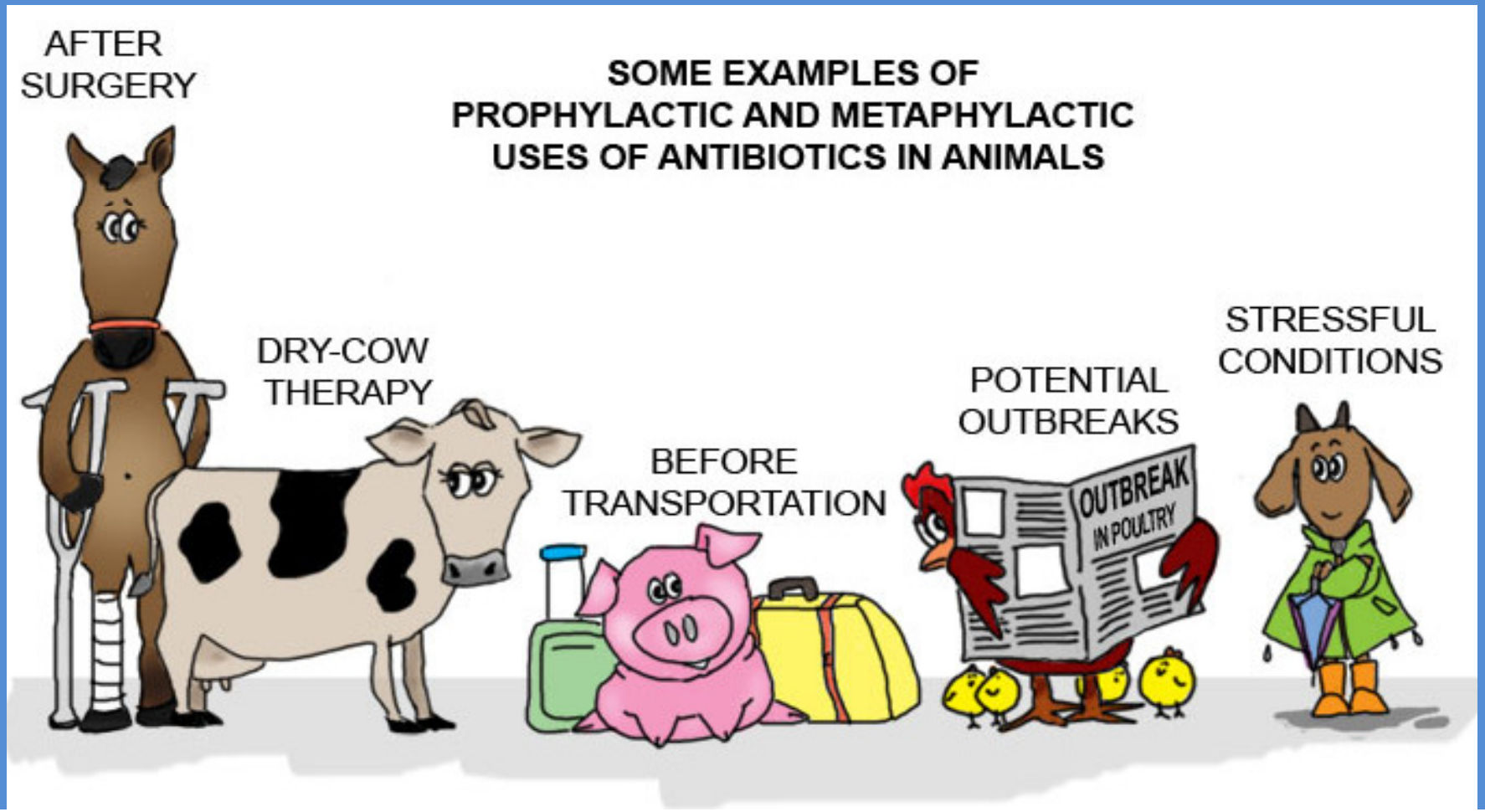
- Can effectively inactivate microbial cells such as: *Hafnia alvei, E. coli, Staphylococcus aureus, Staphylococcus epidermidis, Leuconostoc mesenteroides and Saccharamoyces cerevisiae*

- Antimicrobial activity of PAW against four types of bacterial strains E.col, K. pneumoniae, Acinetobacter baumanii and P. aeruginosa

After 20 minutes of treatment it reduces the microbial load of E.coli and A.baumanii

After 30 minutes of contamination with PAW it determines the complete inactivation of the four strains.

Some alternatives have negative consequences for public health:

For example, supplementation with trace elements, e.g. Zn and Cu, as an alternative to swine colibacillosis control (Fairbrother, 2005; Hojberg 2005), significantly increased the proportion of multidrug-resistant *E. coli* in vivo in the enteral microbiome of pigs (Bednorz, 2013) and increased methicillin resistance in staphylococci. (Yazdankhah, 2014).

Thank you for your attention!